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The Dynamics of Human and Rattlesnake Conflict in Southern California

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The Dynamics of Human and Rattlesnake Conflict in Southern California

by

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DEDICATION

To God for sustaining me through difficult times.

To my mother for instilling in me a love of the outdoors.

To my father for cultivating in me a fascination with biology.

To my children, Madeleine, Nathalie, and Ethan, who continue to inspire me and motivate me to do my best.

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ABBREVIATIONS

ACP	Antivenin (Crotalidae) Polyvalant
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CRiSP	Cysteine-rich Secretory Protein
DFA	Discriminant Function Analysis
ED_{50}	Median Effective Dose
HPLC	High-pressure Liquid Chromatography
ICC	Intraclass Correlation Coefficient
iSSS	Initial Snakebite Severity Score
LCH	Local Convex Hull
LD_{50}	Median Lethal Dose
LDT	Long Distance Translocation
MANCOVA	Multivariate Analysis of Covarience
MCP	Minimum Convex Polygon
mSSS	Maximal Snakebite Severity Score
NT	Not Translocated
RL	Range Length
RP	Reverse Phase
SDT	Short Distance Translocation
SHM	Significant Human Modification
SSS	Snakebite Severity Score
SSsS	Snakebite Severity Subscore
SVSP	Snake Venom Serine Protease

ABSTRACT OF THE DISSERTATION

The Dynamics of Human and Rattlesnake Conflict in Southern California

by

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Dr. William K. Hayes, Chairperson

Human-rattlesnake conflict occurs when rattlesnakes are discovered in human-dominated areas and are deemed to pose an unacceptable risk to humans because of their venomous bite. In this dissertation, I investigated the nature of this conflict from the perspectives of both the behavioral and survival risks posed to rattlesnakes and the medical risks posed to humans. In the first of three studies, I investigated the effects of short- and long-distance translocation (SDT and LDT) of nuisance wildlife as a way of mitigating conflict between humans and naturally occurring Red Diamond Rattlesnakes (*Crotalus ruber*) near residential development in southern California. Snake activity ranges and risk of moving near human-modified areas were larger for LDT and SDT snakes than for non-translocated snakes. Snakes moved closer to human-modified areas and required translocation more often during the summer. Snakes translocated greater distances were less likely to return to human-modified areas, and translocation did not affect snake survival. In the second study, I investigated the etiology and severity of human envenomations using a retrospective review of 354 snakebite cases admitted to Loma Linda University Medical Center between 1990 and 2010. Male snakebite victims and those using alcohol or drugs were more likely to sustain

bites to the upper extremity, distal to the ankle or wrist, and via illegitimate provocation of the snake. Snakebite severity was positively associated with snake size, negatively associated with patient mass, and independent of patient age, snake taxon, anatomical location of bite, legitimate versus illegitimate (provoked) bites, and time until hospital admission. Effectiveness of CroFab antivenom was similar for all southern California venomous snake taxa. In the final study, using the same medical data, I assessed the usefulness of several factors as predictors of overall snakebite severity, symptom progression, and antivenom use. Initial snakebite severity score, size of the envenoming snake, and patient mass were significant predictors. I suggested several rules of thumb that could help clinicians anticipate antivenom needs. Overall, this dissertation contributes to our understanding of the effects of mitigation translocation on rattlesnakes and the epidemiology and clinical management of venomous snakebite in southern California.

CHAPTER ONE

INTRODUCTION

Human-Wildlife Conflict

To say that humans and wildlife are in conflict is somewhat of an understatement. We, as humans, dominate almost every ecosystem on the planet (Vitousek et al. 1997). We directly impact between 75–83% of the Earth's land (Sanderson et al. 2002; Ellis & Ramankutty 2008) and significantly impact 96% of the world's oceans (Halpern et al. 2008). Our impact has generally not been a positive one with 338 known vertebrate extinctions since the year 1500 (Ceballos et al. 2015) and many more species experiencing serious population declines (Butchart et al. 2010) largely due to anthropogenic forces like habitat destruction, overexploitation, pollution, human war and conflict, and global climate change (Chivian & Bernstein 2008).

However, the ways in which humans negatively impact wildlife populations is generally not what is meant by the term human-wildlife conflict in the scientific literature. Rather, the reverse is meant with the term largely referring to situations were wildlife negatively impacts (or has the potential to impact) humans (Peterson et al. 2010). Different species may affect humans in different ways, however, common motifs involve herbivores impacting food crops, carnivores impacting human safety, or meso-mammals (e.g. raccoons) causing property damage (Peterson et al. 2010). The term human-wildlife conflict itself has been criticized because, given the definition the term conflict, it implies the incorrect notion of wildlife as a conscious human antagonists. The real conflict, it is argued, is between human groups who may advocate competing agendas, for example, groups advocating species conservation vs. those attempting to expand or preserve livelihoods or economic interests. Thus, human-wildlife conflict involves two aspects—human-wildlife impacts, which involve the negative impacts of wildlife on humans, and human-human conflicts between those wishing to conserve and protect wildlife and those with competing interests and attitudes (Peterson et al. 2010; Young et al. 2010; Redpath et al. 2014).

Human-Rattlesnake Conflict: Snakebite

Both of these aspects of human-wildlife conflict are evident in the case of rattlesnakes. The human-wildlife impact of rattlesnakes fits the common motif of a carnivore impacting human safety. In United States, rattlesnakes often come in contact with people (Nowak & Riper 1999; Mccrystal & Ivanyi 2008) and even though public perception of the risk posed by venomous snakes (including rattlesnakes) may be inflated (Hardy et al. 2001; Gibbons & Dorcas 2002), they do represent a legitimate medical risk. Rattlesnake envenomation can cause potentially life-threatening hematotoxicity and neurotoxicity, as well as significant local soft tissue damage that can result in long-term physical and emotional morbidity (Dart et al. 1992; Smith & Bush 2010; Williams et al. 2011) and significant financial cost (Corneille et al. 2006). However, in the U.S. and Canada, this risk is mitigated by a fully modernized health care system. Although some 2,683–3,858 venomous snake envenomations occur annually in the U.S., only 5–7 deaths are reported (Kasturiratne et al. 2008).

Many factors can influence the risk of snakebite, including those that relate to the snake and those that relate to the human (Hayes & Mackessy, 2010). In terms of the snake, hospital-based studies have consistently shown that larger snakes tend to cause more severe bites (Wingert & Chan 1988; Hayes et al. 2005; Janes et al. 2010; see also

Hackett et al. 2002) because they inject larger quantities of venom (Hayes 1991; Hayes 2008). However, the idea that more provoked snakes deliver more venom, and therefore a more severe bite, has had mixed support, especially for rattlesnakes (Herbert 1998; Rehling 2002). Venom composition, which varies with ontogeny and among populations and taxa, also affects clinical severity (Hayes & Mackessy 2010; Massey et al. 2012). In terms of the human victim, evidence supporting the suggestion that smaller patients tend to have more severe bites has also been mixed. While some studies have supported this contention (Hayes et al. 2005; Pinho et al. 2005), others have failed to detect this relationship (Parrish et al. 1965; Janes et al. 2010). Bite severity might also be influenced by site of the bite, dictated largely by human behavior (Wingert & Chan 1988; Moss et al. 1997; Tanen et al. 2001); presence of clothing (Herbert & Hayes 2009); general health (Tanen et al. 2001; Benítez et al. 2007; Ribeiro et al. 2008); delay to treatment (Pinho et al. 2005; Michael et al. 2011; Paul & Dasgupta 2012; Saravu et al. 2012); and the treatment itself.

Physicians who treat snakebite have varying levels of education and experience regarding proper treatment. For the most part, they must rely on expert advice provided during their course of training or continuing education. Many rely entirely on one or two key sources of information, which include: 1) the product package insert for CroFab or Anavip, the two currently approved medications (antivenoms) for treatment of North American viperid envenomations; and 2) authoritative reviews that provide an algorithm for treatment, such as Lavonas et al., (2011). These sources of information are generally based on the best available clinical research. However, advances continue to be made in snakebite treatment, and room exists for improvement.

One key research tool has been the development of the snakebite severity score (SSS). This scoring method was developed by Dart et al. (1996) and scores bite severity from 0–20 points based on the objective evaluation of clinical parameters in six categories: local wound effects, hematologic (coagulation) parameters, and symptoms associated with the pulmonary, cardiovascular, gastrointestinal, and central nervous systems. The scores for each of these categories, which range from 0–3 or 0–4 are evaluated separately and then summed to obtain a final score. Higher SSS scores indicate a more severe bite. This tool has been used to glean important information regarding treatment options, and to provide a better understanding of the factors that contribute to snakebite severity. As examples, SSS has been used to assess the effect of anatomical bite location on clinical severity (Moss et al. 1997), to assess the effectiveness of CroFab in children (Offerman et al. 2002), to assess the effects of the size of the envenoming snake on bite severity (Janes et al. 2010) and recently to compare the incidence of late coagulopathy in patients treated with CroFab versus the recently FDA approved antivenom (Anavip; Bush et al., 2015).

Historical Attitudes toward Rattlesnakes

The human-human conflict surrounding rattlesnakes is also evident in the opposing attitudes of those who desire to conserve and protect rattlesnake species and those who have a deep-seated, visceral animosity towards snakes in general and venomous snakes in particular. Venomous snakes and humans have had a complex relationship throughout recorded history. In many respects, the ways that humans have interacted with and impacted snakes has been similar to our interactions with other species. Venomous snakes have been impacted by many anthropogenic forces, including habitat destruction and fragmentation, hunting pressure, harvesting for food and traditional medicine, and climate change (Gibbons et al. 2000; Dodd 2001; Böhm et al. 2013). Yet, the dynamics of the human-snake relationship have also been profoundly affected by human attitudes and culture—perhaps to a greater degree than many other species (Mundkur 1983). Snakes seem to elicit strong emotional reactions in people. Evidence from the field of psychology has suggested that, while the fear of snakes is learned, it can be learned more quickly than fears to other things (Öhman & Mineka 2003). This may explain why ophidiophobia (the irrational fear of snakes) is common in the United States (Agras et al. 1969).

However, irrational fear is not the only way these deep emotional reactions to snakes have been expressed throughout history. Many cultures have had profound reverence for and even worshiped snakes (Mundkur 1983). This can be seen in the culture of the Native American peoples and their attitudes toward rattlesnakes (Sasaki et al. 2008). Many Native American tribes had taboos against killing rattlesnakes, believing the snakes had supernatural powers and would harm humans (via their venom or natural disaster) if not treated with respect. The Hopi, for example, believed that rattlesnakes could control the weather, and that periods of drought resulted because they had abused rattlesnakes (Sasaki et al. 2008). Other tribes considered rattlesnakes to be allies. The Mohicans and the Delawares called them "grandfather," with some believing that rattlesnakes could act as guardians by warning them of danger with their rattle (Sasaki et al. 2008).

Unfortunately, this same level of respect was not shared by those who colonized the Americas, particularly from Europe. These people tended to think of rattlesnakes as dangerous vermin that interfered with their way of life. This attitude may have been exacerbated by the fact that many of these colonists were Christian and, based on certain passages in the Bible (i.e. Genesis 3:1; 3:14), associated snakes with the Devil and the fall of humanity into sin (Sasaki et al. 2008). Regardless of the origins of these attitudes, these colonists subscribed to the idea that the only good rattlesnake was a dead one, and killed any they found. This lead to organized efforts to eradicate rattlesnakes, beginning in the early 1700s (Sasaki et al. 2008). These attitudes persist to the present, and are nowhere more evident than at modern rattlesnake roundups, the largest of which (the Sweetwater Jaycees Rattlesnake Round-up in Texas) indiscriminately slaughters as many as 18,000 snakes in a single weekend event (Weir 1993; Sasaki et al. 2008).

Contemporary Attitudes toward Rattlesnakes

These attitudes of fear and prejudice continue to play out when humans and rattlesnakes come into conflict in North America. The fact that rattlesnakes possess a medically significant venomous bite does not help their image. However, there are many, including scientists and conservationists, who recognize the intrinsic beauty and value in these animals. Despite the risks they pose to humans, rattlesnakes comprise an important part of the ecosystems to which they belong. They are often top-order predators in the habitats they occupy. Thus, even the loss of a few snakes from the population could result in significant ecological consequences (Shine & Koenig 2001; Estes et al. 2011; Sullivan et al. 2015). Some of these consequences could have direct negative impacts on humans. For example, destabilization of ecosystems and reduction of biodiversity has been suggested to put humans at increased risk of zoonotic diseases (Keesing et al. 2006; Ostfeld & Keesing 2014). Rattlesnakes may play a role in that balance by consuming rodents, which can be disease vectors. Indeed, one study has suggested that Timber Rattlesnakes (*Crotalus horridus*) reduce the incidence of Lyme disease in the northeastern U.S. by helping to control small mammal populations (Kabay 2013). Thus, negative attitudes toward snakes in general, and rattlesnakes in particular, can not only impede conservation (Dodd 1993) but may also impact human health.

Mitigation of Human-Rattlesnake Conflict

However, determining the best action to take when human-rattlesnake conflict occurs is not always clear. Middle ground must be found between those who would emphasize public safety and those who wish conserve and protect rattlesnake species. Certainly rattlesnakes pose some risk to humans but, from the perspective of the conservationists, human-rattlesnake conflicts can also be of great risk to the snake. When humans encounter a rattlesnake in the wild, one of two things can happen, depending on what the human decides. The decision could be to avoid further contact with the rattlesnake, and leave it alone, or to deal somehow with the rattlesnake. The human decision to avoid the snake and leave it alone is certainly the best option from the perspective of the snake. Given the previously mentioned attitudes and biases many people have, humans often decide against leaving the snake alone, which often results in killing of the snake. Human-rattlesnake conflicts that occur on private residential property may place the snake at even greater risk. In this situation, the human may feel that leaving the snake alone is not a viable option because the snake poses too great a risk to them or their family if it remains on their property. In many cases, a property owner may decide to kill the snake, though a wildlife management professional may also be called in to remove the offending snake (Mccrystal & Ivanyi 2008). Understanding the dynamics of human-rattlesnake conflict and the risks to both humans and snakes is essential for managing conflicts and risks, and is vital to inform conservation efforts for the snakes.

Mitigation Translocation

In general, animals removed from conflict with humans are generally dealt with in one of two ways—euthanization or mitigation translocation (Craven et al. 1998). Mitigation translocation is defined as the action of moving nuisance wildlife to areas where they are no longer in conflict with humans (Sullivan et al. 2015). The public often supports this option over euthanization because it is considered more humane (Massei et al. 2010). However mitigation translocation can have negative consequences for the translocated animal. Survival for translocated animals requires that they be able to find and secure critical resources (i.e. mates, food, shelter) in an unfamiliar environment while competing for these resources with resident conspecifics and avoiding predation (Massei et al. 2010). This is a difficult task and may result in translocated animals exhibiting erratic movement patterns and suffering high mortality rates and, at least in the short term (Massei et al. 2010; Sullivan et al. 2015). How well an animal deals with the stress of translocation depends, to some degree, on species ecology. Omnivores are often more likely to survive after translocation than herbivores and more generalist herbivores are often adapt more quickly than carnivores (Griffith et al. 1989; Massei et al. 2010).

Beyond the effects of translocation on the individual animal, mitigation translocation may negatively affect resident animals in the areas where translocated animals are moved to. Translocated animals may introduce disease, disrupt social structure, or contribute to outbreeding depression (Burke 1991; Reinert 1991; Chipman et al. 2008; Massei et al. 2010; Sullivan et al. 2015). Moreover, mitigation translocation may not always be effective (Fischer & Lindenmayer 2000). Translocated animals may continue to come in conflict with humans, either by returning to the original conflict area (Cunningham 1996; Linnell et al. 1997; Massei et al. 2010; Sullivan et al. 2015), or by moving to new conflict areas after translocation (Linnell et al. 1997; Massei et al. 2010). One study that involved mitigation translocation of leopards in India found an opposite effect of what was intended, with translocated leopards posing more of a risk to humans that non-translocated ones (Athreya et al. 2011).

Concerns regarding mitigation translocation center largely on the distance that the nuisance animal is moved. Animals moved a short distance suffer fewer consequences but are more likely to return to areas of conflict, whereas animals moved a longer distance are less likely to return but may suffer higher rates of mortality and contribute to population-level problems (Massei et al. 2010). Some studies define translocation categories relative to the home range size of the animal, with short-distance translocation (SDT) being within the animal's normal home range, and long-distance translocation (LDT) extending beyond the home range but usually within the local breeding population or deme (Hardy et al. 2001). These distances can vary from several hundred meters for reptiles and small mammals (e.g., McGregor et al. 2008) to many kilometers for larger mammals (e.g., Athreya et al. 2011). Translocations of much greater distances, beyond the local deme, have been referred to as regional or intra-continental translocation (Loss et al. 2011).

Overall, many variables must be taken into consideration when deciding the best course of action when dealing with human-wildlife conflict. In many cases the human interests involved (crops, safety, property, etc.) must be respected and the animal causing the impact must be removed. Deciding whether euthanization or mitigation translocation is the best option requires assessing the human and ecological dimensions on a species by species or case by case basis.

Mitigation Translocation of Nuisance Rattlesnakes

Human-rattlesnake conflict is a case in point. At present, only a limited number of studies have examined human-wildlife conflict related to rattlesnakes. These studies have been limited to looking at the effects of translocation on the translocated snakes themselves with no studies looking at the effects of translocation on conspecifics in area snakes are translocated into. As with translocation in other species, most studies reveal that translocated rattlesnakes tend move more and have more erratic movement patterns than untranslocated snakes (Reinert & Rupert 1999; Nowak et al. 2002; Brown et al. 2008; Brown et al. 2009). However, with respect to mortality, studies have been mixed. Increased mortality has been cited as major reason why mitigation translocation in snakes fails (Sullivan et al. 2015). However, while some studies have shown a significant increase in the mortality of translocated rattlesnakes (Reinert & Rupert 1999; Nowak et al. 2002), others have not (Brown et al. 2008; Brown et al. 2009). Some studies have also looked at the effectiveness of SDT vs LDT in mitigating human-rattlesnake conflict. Despite one study that concluded that SDT was effective (Sealy 2002), most others report that SDT snakes often return to conflict areas (Hardy et al. 2001; Brown et al. 2008; Brown et al. 2009) therefore may not ultimately solve the problem that translocation was intended to resolve.

Given the potential problems with translocation some may consider euthanization as the best option. However, indiscriminant use of euthanization is also problematic. As mentioned previously, rattlesnakes are integral members of their ecological communities, and disruption of these communities may result if even a few rattlesnakes are removed. Euthanization may also be problematic when the nuisance species is endangered or protected. Euthanization may run counter to conservation efforts, and may be contrary to legal mandates that require maintenance of protected lands and the species they contain (Nowak & Riper 1999).

Additional research is sorely needed to better understand the impact of mitigation translocation on rattlesnake ecology, particularly in terms of mortality and the impacts of translocation on conspecifics in areas where snakes are translocated into. It is only by having a thorough understanding of the impacts of our mitigation strategies on the dynamics of human-rattlesnake conflict that we can we make informed decisions that are in the best interests of both rattlesnakes and humans.

Specific Objectives

In this dissertation, I begin, in chapter 2, by examining the effectiveness of translocation as a strategy to mitigate-human rattlesnake conflict and the effects of translocation on the snakes themselves. I studied the effect of short-distance translocation (SDT) and long-distance translocation (LDT) on Red Diamond Rattlesnakes (*Crotalus ruber*) located near a residential development in Southern California using radiotelemetry. I also examined sexual and seasonal differences in movement patterns and space use by the snakes, and how these relate to residential development.

In Chapter 3, I examine the major way in which rattlesnakes negatively impact humans, particularly in relation to their venomous bite. I describe the results of a retrospective review of the medical records of snakebite victims at the Loma Linda University Medical Center. This study looked at factors related to the etiology and clinical severity of rattlesnake envenomations in southern California. These factors included the species and size of the snake, the mass and the anatomical location of the bite on the victim, the type of interaction the victim had with the snake (legitimate or illegitimate), and the time between the bite and the victim's arrival at the hospital. This study also examined when, during the year and time of day, human-rattlesnake interactions resulting in snakebite were most likely to occur.

In Chapter 4, I continue to examine human envenomation by rattlesnakes, this time looking at it from the perspective of a clinician. Using the same medical record data as the previous chapter, I examine whether assessing several factors related initial presentation at the hospital could predict symptom progression and the amount of antivenom needed to resolve symptoms. These factors were the same as in Chapter 3, including the species and size of the snake, the mass of the victim and the anatomical location of the bite, the type of interaction the victim had with the snake (legitimate or illegitimate), and the time between the bite and the victim's arrival at the hospital.

In chapter 5, I summarize and discuss the results from my research as a whole. My findings should provide a clearer picture of the nature of human-rattlesnake conflict in southern California. They should aid those involved with wildlife management in making decisions about how to mitigate human-rattlesnake conflict, and they should benefit clinicians in treating snakebite victims.

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CHAPTER TWO

RATTLESNAKES ON THE EDGE: THE EFFECT OF LONG- AND SHORT-DISTANCE TRANSLOCATION ON THE MOVEMENT PATTERNS OF RED DIAMOND RATTLESNAKES (*CROTALUS RUBER*) IN CONFLICT WITH HUMAN RESIDENTIAL DEVELOPMENT

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Abstract

Mitigation of human-rattlesnake conflict generally involves euthanizing or translocating the offending rattlesnake. Of these, translocation is generally considered more humane, especially by the general public. However, it may significantly impact the individual snake that is translocated. We studied the effect of short-distance translocation (SDT) and long-distance translocation (LDT) on Red Diamond Rattlesnakes (*Crotalus ruber*) located near residential development in Southern California. Depending on measure (minimum convex polygon, local convex hull, range length), activity ranges of LDT snakes were 38.6–67.1% larger than those of SDT snakes, which, in turn, had activity ranges that were 77.0–152.9% larger than those of non-translocated (NT) snakes. Snakes moved closer to human modified areas during the summer, and were translocated most often during that season at the behest of property owners. Analysis using Cox regression revealed that both SDT and LDT snakes were more likely to move into human-modified areas subsequent to translocation than NT snakes. For translocated snakes, every 1 m increase in distance moved resulted in a 1.2% decreased risk of moving into a human-modified area, and a 1.5% decreased risk of returning to the site of capture. We found no differences in the survival rate between translocated snakes (LDT an1d SDT) and NT snakes. Our findings suggest that LDT of nuisance snakes may be a viable option for at least some rattlesnake species. To reduce confusion arising from different meanings of the terms SDT and LDT among different studies, we propose standardizing the terms for distance of movement as alpha- (within the individual's home range), beta- (within the local deme), gamma- (beyond the local deme), and delta-translocation (to regions unoccupied by the species, including inter-continental).

Introduction

As human residential and commercial development encroaches upon natural areas, human interactions and conflicts with native wildlife inevitably increase (Conover 2002; Rosie Woodroffe et al. 2005). In many cases, these interactions are positive, providing a basis for public interest in native species and their conservation. However, many of these interactions are also undesired, with wildlife either causing property damage or injury to humans, or being perceived as a significant risk of causing such. To mitigate human-wildlife conflict, two options may be used: 1) limit human access to areas were conflict may occur; or 2) remove the offending animals. Limiting human access to potential conflict areas is often the best choice, as it can minimize disturbance to the animals and the risks to the public. It has also been shown to be effective (Fernández-Juricic et al. 2004; Benn & Herrero 2014; Carter et al. 2014). However, this method is predicated on identifying specific areas with a significant increased risk of conflict, and having the legal authority to limit human access to the area. Yet there are many situations where these two criteria cannot be met, either because no specific areas of increased risk can be identified, or because the legal authority to restrict human access cannot be obtained. The latter issue is particularly acute when nuisance wildlife moves onto private property, as the legal authority to mitigate the conflict by limiting a person's access to their own property generally cannot be obtained. In such cases, removing the offending animal is the only option apart from doing nothing.

Animals removed from conflict with humans are generally dealt with in one of two ways: euthanization or translocation (Craven et al. 1998). Translocation simply refers to the movement of one or more organisms from one place to another, and has been used as an overarching term that covers a number of wildlife management practices, including establishing, reestablishing, or augmenting populations for conservation purposes (Griffith et al. 1989; Reinert 1991; IUCN/SSC 2013).

Mitigation translocation, defined as the action of moving nuisance wildlife to areas where they are no longer in conflict with humans (Sullivan et al. 2014), is often preferred over euthanization, particularly by the public, which views it as more humane (Massei et al. 2010). However, many studies examining the effects of translocation have urged caution based on three potential concerns. First, translocated animals often suffer high mortality rates, and exhibit erratic movement patterns, at least in the short term (Massei et al. 2010; Sullivan et al. 2014). Second, mitigation translocation may not always be effective (Fischer & Lindenmayer 2000), as translocated animals may continue to come into conflict with humans, either by returning to the original conflict area (Cunningham 1996; Linnell et al. 1997; Massei et al. 2010; Sullivan et al. 2014), or by moving to new conflict areas after translocation (Linnell et al. 1997; Massei et al. 2010). Third, translocated animals may negatively affect conspecifics (or even heterospecifics) in the areas they are moved to by introducing disease, disrupting social structure, or contributing to outbreeding depression (Burke 1991; Reinert 1991; Chipman et al. 2008; Massei et al. 2010; Sullivan et al. 2014).

These concerns regarding mitigation translocation center largely on the distance that the nuisance animal is moved. Animals moved a short distance suffer fewer consequences but are more likely to return to areas of conflict, whereas animals moved a longer distance are less likely to return but may suffer higher rates of mortality and contribute to population-level problems (Massei et al. 2010). Unfortunately, discussion of the merits and concerns of translocation is sometimes confounded by spatial scale, a problem arising, in part, because no established terminology exists for translocation distance. Some studies arbitrarily define translocation categories relative to the home range size of the animal, with short-distance translocation (SDT) being within the animal's normal home range, and long-distance translocation (LDT) extending beyond the home range but usually within the local breeding population or deme (Hardy et al. 2001). These distances can vary from several hundred meters for reptiles and small mammals (e.g., McGregor et al. 2008) to many kilometers for larger mammals (e.g., Athreya et al. 2011). Translocations of much greater distances, beyond the local deme, have been referred to as regional or intra-continental translocation (Loss et al. 2011). Obviously, the consequences of moving an animal into a neighbor's home range versus a distant region can differ dramatically, yet both have come under the rubric of "long-distance translocation." A more universal terminology, such as alpha- (within the individual's home range), beta- (within the local deme), gamma- (beyond the local deme), and delta-translocation (to regions unoccupied by the species, including inter-continental), could help clarify the discussion of problems associated with translocation. In this paper, we will use SDT to refer to alpha-translocation and LDT to refer to beta-translocation, unless otherwise indicated, but we urge researchers to adopt a more formalized rubric for the spatial scale of translocation.

In the United States, venomous snakes often come in conflict with humans (Nowak & Riper 1999; Mccrystal & Ivanyi 2008). Even though public perception of the risk posed by venomous snakes may be inflated (Hardy et al. 2001; Gibbons & Dorcas 2002), they do represent a legitimate health risk. Though mortality in the U.S. is low (5–7 deaths annually), venomous snakebite is still a significant medical issue, with an estimated 2,683–3,858 envenomations annually (Kasturiratne et al. 2008) that incur substantial costs and physical and emotional morbidity (Dart et al. 1992; Corneille et al. 2006; Smith & Bush 2010). Because of this, there is some public support for euthanizing nuisance venomous snakes (Braband & Clark 1991).

However, indiscriminant use of euthanization is problematic. Venomous snake are often top-order predators in many of the habitats they occupy; hence, even the loss of a few snakes could have significant ecological consequences (Shine & Koenig 2001; Estes et al. 2011; Sullivan et al. 2014). Euthanization may also be problematic when the nuisance species is endangered or protected. Euthanization may run counter to conservation efforts, and may be contrary to legal mandates that require maintenance of protected lands and the species they contain (Nowak & Riper 1999).

Yet the same issues exist with mitigation translocation in venomous snakes as with other species, generally including erratic movements and increased mortality. Because shorter distances may negate the negative effects of translocation (Massei et al. 2010), it has been suggested that nuisance rattlesnakes can be effectively managed using SDT (Sealy 2002) rather LDT (beta- or gamma-translocation), with the latter often employed in an attempt to ensure that the snake will not return to the area of conflict (Nowak et al. 2002). Despite one author reporting success with SDT (Sealy 2002), others report that SDT snakes often return to conflict areas (Hardy et al. 2001; Brown et al. 2008; Brown et al. 2009), and therefore may not ultimately solve the problem that translocation was intended to resolve.

Despite the fact that several studies have examined translocation in rattlesnakes, many have methodological weaknesses. Several such studies utilized radiotelemetry to monitor venomous snakes that were translocated in order to establish or reestablish populations for conservation purposes (Johnson 1993; Hare & McNally 1997; King et al. 2004; Walker et al. 2009; Harvey et al. 2014). While such studies provide valuable information on the behavior of translocated snakes, these studies lacked non-translocated control snakes. Other studies have utilized mark/recapture methods (Hardy et al. 2001), though these are poorly suited for determining snake mortality and how often snakes return undetected to conflict areas. Studies that have utilized radiotelemetry and appropriate control groups are summarized in Table 1. Most of these studies show an increase in movements and the size of activity ranges in both SDT and LDT (alpha- and beta-translocation) snakes. Some studies have further concluded that LDT (beta- and gamma-translocation) snakes experience increased mortality (Reinert & Rupert 1999; Nowak et al. 2002), whereas others have not (Brown et al. 2008). To date, no studies have effectively compared both LDT and SDT snakes within the same study. Brown et al. (2008) came close in studying both LDT and SDT Red Diamond Rattlesnakes (*Crotalus ruber*); however, deaths of two of three SDT snakes due to surgical complications precluded statistical analysis between these groups.

Like Brown et al. (2008), we examined the effects of translocation on Red Diamond Rattlesnakes in southern California. This snake is a species of special concern in the state because of habitat loss due to human development (Jennings & Hayes 1994), and has recently received formal protection from collection and possession. As such, euthanization may not be desirable as an option for mitigating human-wildlife conflict caused by this species. The aim of this study was to examine the effects of both long- and short-distance translocation in this protected species in order to inform future policies for dealing with nuisance rattlesnakes. Our study represents, to our knowledge, the only study to date that is able to compare LDT, SDT, and untranslocated rattlesnakes within a single study.

Table 1. Summary of mitigation translocation studies in rattlesnakes that employed radio-telemetry and included a control group.[a] Effect size for survival differences is Cramer's *V*. Activity range and movement differences relative to controls are indicated as increases (+) or no difference (0). Other effects refer to translocated snakes.

		Control Group		Translocation Group							
Species	**Duration (months)**	**N**	**Survival (%)**	**Type**	**N**	**Survival (%)**	**Effect Size**	**Activity Range**	**Movements**	**Other Effects**	**Source**
C. atrox	28	6	83.3	LDT	7	57.1	0.283	+	+		Novak et al. 2002
C. horridus	48	18	88.9	LDT	11	45.5	0.472	+	+		Reinert & Rupert 1999
C. o. oreganus	18	14	100.0	SDT	14	85.7	0.277	0	+		Brown et al. 2009
C. o. oreganus	2	14	100.0	SDT	8	100.0	0.000	+	0	Larger medial cortex	Holding 2011, 2012, 2014
C. o. oreganus	1	7	100.0	LDT	7	100.0	0.000	+	0	Visible more often; greater levels of testosterone	Heiken 2013[b]
C. ruber	60	11	90	SDT/LDT	6	75	0.194	+	+		Brown et al. 2008[c]
C. ruber	37[d]	10	60	SDT/LDT	12/10	63/89	0.23	+	+		This Study[e]

[a] Deaths due to surgical complications are excluded; survival calculations exclude missing snakes (often due to transmitter failure) as mortalities.
[b] One mortality that occurred prior to translocation is excluded
[c] Two of the three SDT snakes in this study died due to surgical complications, making comparison between LDT and SD snakes impossible.
[d] Maximum duration a single snake was tracked
[e] Human caused mortality excluded

Materials and Methods

Study Site

The study was conducted in the southern portion of Loma Linda, California (34°02' N, 117°16' W), within a ca. 500-ha boundary area between human development to the north and a largely undeveloped area of rolling hills (ca. 324 km^2) that extends southeast (Figure 1). The portion containing natural habitat consists largely of non-native grassland, though steeper north-facing slopes are covered by coastal sage scrub dominated by California Sagebrush (*Artemisia californica*) and Black Sage (*Salvia mellifera*), and many south-facing slopes are populated by Brittlebush (*Encelia farinosa*). The site lacks the rock outcrops and substantial cactus patches that are generally preferred by *C. ruber* (Dugan et al. 2008; Halama et al. 2008). Human development in this area largely consists of a residential area containing homes, sprinkler-irrigated lawns and gardens, and non-native tree species.

The area also contains some small citrus orchards on the eastern end and a large cemetery on the western end that includes large trees and a lawn kept green year-round by sprinkler irrigation. The site experiences a Mediterranean climate (Cowling et al. 1996), with much of the ca. 40-cm average annual precipitation occurring during winter and spring. Winters are mild, with the mean daily low temperature in January being 5.6°C, whereas summers are hot and dry, with the mean daily high in July being 35.7°C (Western Regional Climate Center n.d.).

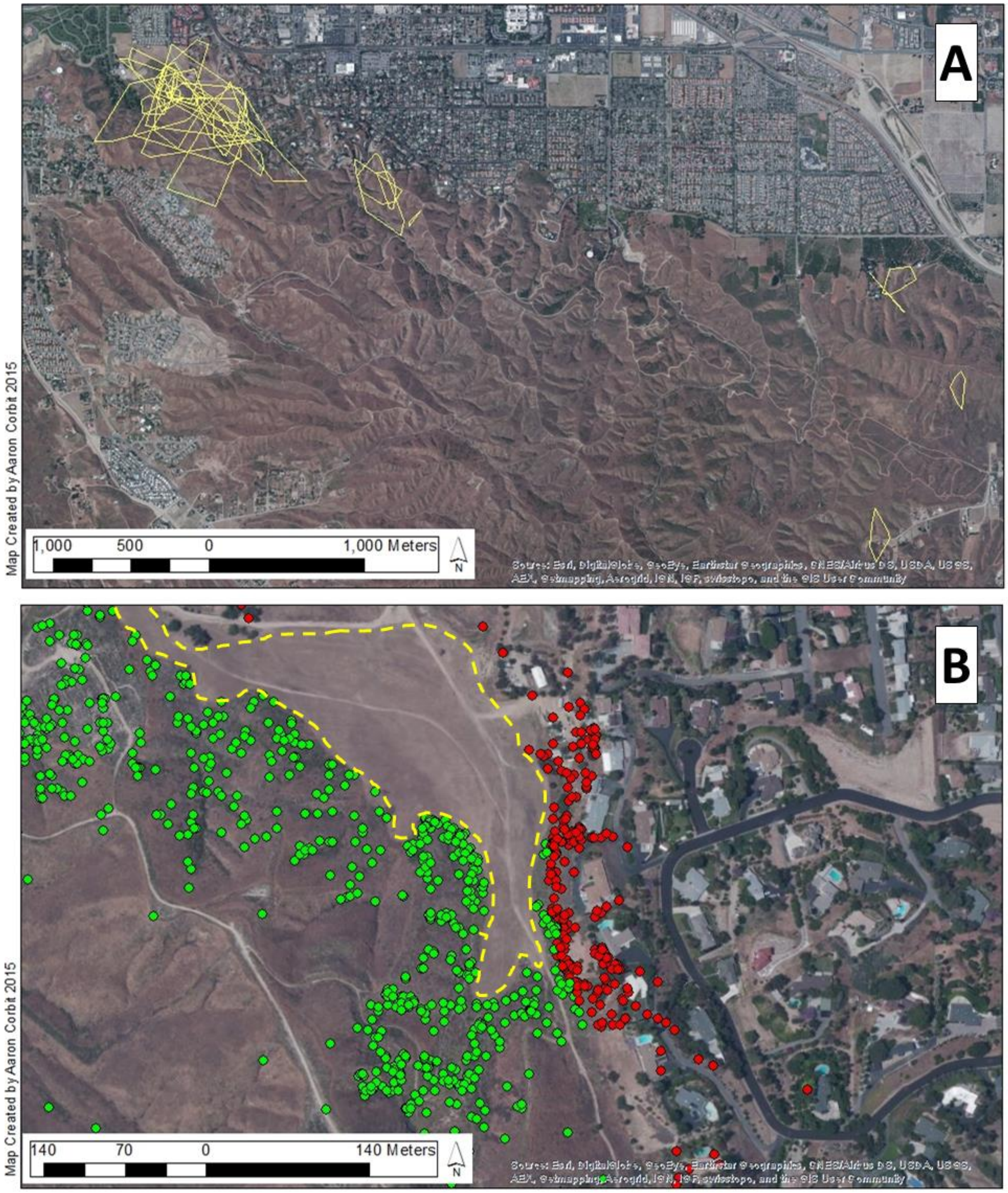

Figure 1. (A) Study site in Loma Linda, California, USA, showing 100% Minimum Convex Polygons for all fixes of all radiotelemetered Red Diamond Rattlesnake (*Crotalus ruber*) in the study. (B) Spatial depiction of a western portion of the study site. Radiotelemetry fixes (all snakes pooled) are shown for natural habitat (green circles) versus human-modified areas (red circles). Yellow dashes outline a plowed firebreak that snakes moved across but never stopped within.

Legal Issues

The study required cooperation from local residents, who reported snakes to us and allowed access to their property. To solicit their help, we distributed fliers to those whose property bordered natural habitat. Because our study involved potential liability, due largely to the possibility of envenomation from our research subjects, we sought legal advice. We recommend that others contemplating similar studies do the same, so we briefly share the issues we dealt with. Our university's legal counsel researched three risks to the institution, and made the following determinations (with no assurance of legal immunity) from California law: (1) liability would be unlikely for injury caused by a rattlesnake that researchers captured, tagged, released, monitored, and relocated; (2) liability would be unlikely for removal of snakes from an owner's property; and (3) the university could be liable if the homeowner was asked to allow a snake to remain on its property to benefit the research. Accordingly, we drafted a letter to homeowners, approved by legal counsel, which described the study and included a property access agreement. The agreement provided options for (1) access to the property (including time of day and whether permission would be required each time); (2) notification of radio-tracked snake presence on property; (3) whether the snake should be left or removed (all owners requested removal, and we were forbidden to offer advice); (4) details on potentially hostile pets that might be encountered on the property; and (5) release of liability from any damage arising from any snake or researcher involved with the study. Twenty-eight property owners signed the agreement, of which 27 granted us access to their property. We emphasize that our study design was necessarily constrained by these liability issues.

Radio-telemetry

Snake collection, radio-transmitter implantation, and tracking commenced in July 2008 and continued through December 2011. Most snakes were obtained through cooperating residents, who contacted us via telephone when they discovered a rattlesnake in their yard. Snakes were also collected opportunistically when discovered in the field, especially during the spring mating season (February–April; Dugan et al. 2008) when they paired up with telemetered snakes. Once collected, we obtained the snake's mass and total length. Sex was determined by subcaudal scale count (male: ≥24; female: ≤ 23) and/or probing using Neosporin-lubricated sexing probes. Total length was determined via photography in a press-box (Quinn & Jones 1974), with the floor covered by 1-cm graph paper and a clear plastic cover to hold the snake in place. Photographs were imported into ImageJ version 1.47q (Rasband 1997) to calculate snake length.

We used both SI-2 (Holohil Systems Ltd., Ontario, Canada) and SOPB-2190 (Wildlife Materials Inc., Murphysboro, Illinois, USA) radio-transmitters. These transmitters weighed ca. 6 and 9 g, respectively, which always represented <5% of an individual snake's body mass (Hardy & Greene 1999). Surgical procedures followed Reinert & Cundall (1982) and Hardy & Greene (1999). Because minimizing time in captivity reduces post-surgical mortality (Hardy & Greene 1999), snakes were released 24–48 hr after surgery. We obtained fixes on each snake's location 1–2 times weekly using a Telonics TR2 receiver (Telonics, Mesa, Arizona, USA) and a generic four- or six-element yagi antenna. For each fix, we attempted to visually locate the snake and record its coordinates using a handheld GPS unit (Magellan Explorist 210; Magellan, Santa

Clara, California, USA). If a snake was not visible, we took coordinates as close to the source of the radio signal as possible.

Initial Translocation

Subsequent to capture and transmitter implantation, snakes were assigned to one of three groups. Snakes initially found in natural habitat were released at their site of capture and placed in the initially non-translocated control group (NT). Snakes captured in or near human-modified areas were always translocated (by landowner request) to natural habitat away from the area of conflict, and randomly placed in either the initially short-distance translocation group (SDT, <716 m; range 100–600 m) or the long-distance translocation group (LDT, >716 m; range 800–5500 m, with one exception detailed below). At the onset of our study, we adopted the 716-m criterion used by Brown et al. (2008), who distinguished short- and long-distance translocation based on this mean value for the maximum straight-line distance between any two locations for non-translocated *C. ruber* at a study location not far from ours. We made an exception for one female snake which, subsequent to an initial translocation of 519 m, displayed erratic movements away from her capture site, overwintered 662 m from her capture site on the other side of a large hill, and finally returned to within 200 m of her original capture site after 305 days. Given these erratic movements and the fact that female *C. ruber* have significantly smaller home ranges than males (Brown et al. 2008), we interpreted this to mean that we had translocated her out of her normal home range, and therefore assigned her to the LDT group.

Two snakes (1 male and 1 female) in the eastern portion of the study site (where we had access issues) were initially assigned to the SDT group, but were reassigned one year later to the LDT group when they were translocated to the western portion of the study site. These two snakes, subjected to both SDT and LDT treatments and accounted for in the sample sizes noted in Results, were treated as separate snakes in our analyses. This treatment, therefore, constituted minor pseudoreplication across but not within treatment groups; the latter would be much more problematic. Other investigators have made more extensive use in treating movements of the same snake in separate years as independent units (e.g. Plummer & Mills 2000; Harvey & Weatherhead 2006; DeGregorio et al. 2011).

Follow-up Translocation

After implanting transmitters in snakes, and assigning them to a translocation group upon release, we disturbed the snakes as little as possible when tracking their movements via radiotelemetry. However, because of increased risk of injury to humans (and to the snake) as well as for legal reasons, we translocated a short distance (50–400 m) into suitable natural habitat any telemetered animal found in close proximity to a human-modified area. These additional translocations potentially confounded differences between treatment groups (NT, SDT, and LDT); however, our statistical analyses (see below) allowed us to infer differences between groups independent of the effect of follow-up translocations. When snakes near areas of potential human conflict were inaccessible (e.g., beneath wooden patios or concrete slabs) or eluded capture, we alerted the property owner to the snake's presence and returned later to recapture the snake if still necessary. We did not include sites where snakes were released in our activity range calculations, nor did we include the distance the snake was translocated in our calculations of mean daily movement. We attempted to translocate snakes to areas where we had observed them previously, resulting in release sites generally within calculated activity ranges. Because we used non-parametric methods of activity range calculation (see below), which are based on constructing polygons from points at the edge of the snake's activity range, releasing snakes in this way minimized the chances of artificially inflating the calculated area of the activity range.

Activity Range and Movements

We used R statistical software version 2.15.2 (R Core Team 2014) and the package adehabitat version 1.8.12 (Calenge 2006) to calculate several land use and movement parameters. Because several of these snakes were long-distance translocated outside their normal "home" area, we follow Hare & McNally (1997) in using the term "activity range" instead of the more traditional "home range" to describe the area utilized by an animal over a particular time period. Because the snakes were generally inactive during December and January, we used the calendar year for between-year comparisons.

We calculated two activity range statistics for each snake during each season per calendar year of tracking. To facilitate comparison with previous studies, we computed a 100% minimum convex polygon (MCP). Because parametric kernel methods of calculating activity range area, particularly those using least-squares cross-validation to select kernel size, have been shown to be somewhat unreliable for reptiles (Row & Blouin-Demers 2006), we chose to use one of the non-parametric kernel methods known as local convex hull (LCH; Getz & Wilmers 2004; Getz et al. 2007). This method appears to be superior to kernel methods in providing better convergence properties as sample size increases. The LCH is also better able to exclude portions within the activity area that animals never occupied due to physical barriers or undesirable habitats. Anthropogenic alterations at our study sites that blocked snake access or created undesirable habitat included plowed fire breaks and walls erected around some of the residential developments. Three LCH methods have been proposed based on the way the non-parametric kernels are constructed. The “fixed number of points” method (*k*-LCH) constructs kernels from the *k*-1 nearest neighbors of a root point, where *k* is supplied by the user; the “fixed sphere of influence” method (*r*-LCH) constructs kernels based on choosing a fixed radius *r* from each reference point; and the “adaptive sphere of influence” method (*a*-LCH) constructs kernels from all points within a radius *a* such that the distances of all points within the radius to the reference point sum to a value less than or equal to *a* (Getz et al. 2007). Of these three, the *a*-LCH method is considered superior because it provides better area estimates and is more robust to proportional changes in the *a* parameter (Getz et al. 2007), so we chose to use this method. Following the "rule of thumb" in Getz et al. (2007), we set the *a* parameter equal to the range length (RL), which was the maximum distance between any two points for each snake in a given year (i.e., we used an *a* value that was unique for each individual snake). We also used RL as a relative measure of activity range.

To determine the minimum acceptable number of fixes to use for calculating activity ranges, we bootstrapped 10 randomly-selected activity ranges 100 times each, and visually assessed the effect of number of fixes on activity range size. Based on this analysis, we excluded activity ranges of any individual snake with fewer than 20 fixes.

We also calculated mean daily movement by dividing the distance moved between consecutive fixes and the number of days between these fixes (Gregory et al. 2001). These were averaged per snake over each calendar year to get the mean daily movement.

Distance to Human-Modified Areas

For each coordinate fix obtained for each snake, we calculated the distance from that point to the nearest area with significant human modification (SHM). These areas were defined by the presence of buildings, roads, and well maintained gardens and lawns (Figure 1). Areas subject to a lesser degree of human modification, such as plowed firebreaks and dirt trails, were not included. The UTM coordinates of such areas were found using Google Earth software (Google Inc., Mountain View, California, USA), and Euclidean distances were calculated using R software.

Statistical Analyses

We conducted all statistical tests using SPSS v. 13.0 (SPSS Inc., Chicago, Illinois, USA), with alpha set at 0.05. For the analysis of variance (ANOVA) models described below, we computed effect sizes as eta-squared (η^2) for one-way models and partial η^2 for factorial models (Green & Salkind 2005). Effect sizes are independent of sample size (in contrast to statistical significance) and biologically more meaningful, and can be more readily compared among different data sets and different studies (Nakagawa & Cuthill 2007). Eta-squared can be interpreted as percent of variance explained, though partial η^2 becomes upwardly biased as additional independent variables are added to the model (Levine 2002). Values of ~0.01, ~0.06, and ≥0.14 correspond loosely to small, moderate, and large effects, respectively (Cohen 1988). For Chi-square analyses, we computed effect size as Cramer's *V*, with values of ~0.10, ~0.30, and ≥0.50 corresponding loosely to small, moderate, and large effects, respectively (Cohen 1988).

We analyzed data without regard to environmental differences between years. In southern California, one study suggested that Mohave Rattlesnakes (*C. scutulatus*) moved less and occupied smaller home ranges during a dry year compared to two wet years (Cardwell 2008). At our study site, where annual precipitation averages 34 cm (National Climatic Data Center, 2011), the winter rainfall was less preceding the relatively dry 2008 and 2009 seasons (July-June rainfall = 27 and 22 cm, respectively) compared to that preceding the relatively wet 2010 and 2011 seasons (39 and 53 cm). However, the activity ranges and movements of three snakes tracked during both the dry (2008-2009) and wet years (2010-2011), each with ≥20 fixes per year, showed surprisingly consistent home ranges and movements during each year across this time span (data not presented).

Activity Range and Movement Analysis

To examine the effects of initial translocation and sex on annual activity range and movement patterns, we utilized a 2 × 3 (sex × treatment group) factorial ANOVA (Field 2005) for each dependent variable (100% MCP, 100% *a*-LCH, mean daily movement, RL), and included both sex and translocation group as between-subjects factors. We calculated activity range and movement parameters for all snake-year combinations, and then the values for snakes tracked during multiple years were averaged to avoid pseudoreplication (with the aforementioned exception of two snakes subjected to both SDT and LDT). For these analyses, all movement and activity range parameters were rank-transformed to meet parametric assumptions.

We also examined seasonal differences in mean daily movement between sexes using a 2 × 4 repeated-measures ANOVA (Field 2005), with season as a within-subjects factor and sex as a between-subjects factor. Seasons were categorized as spring (March–May), summer (June–August), fall (September–November), and winter (December–February), and mean daily movement was calculated for each season-year combination for each snake. These values were similarly averaged across years for each snake in order to obtain a single value for each snake per season, thereby avoiding pseudoreplication. Snakes that were not tracked over all four seasons were excluded from analysis. We did not transform mean daily movement for this analysis, as parametric assumptions were largely met.

Survival Analysis

We analyzed snake mortality both considering and omitting mortalities due to human causes. Survival rates were calculated using the Kaplan-Meier procedure (Kaplan & Meier 1958), following Pollock et al. (1989) and the guidelines of Robertson & Westbrooke (2005). The time period used in these calculations was the total number of days the snake was tracked. This time period began when the snake was released into the field after transmitter implantation, and ended based on the same last fix criteria used in the Cox proportional hazard analysis. The number of days a snake was held in captivity for surgery or recovery was subtracted from the total time for this analysis. Also, the two snakes that died due to surgical complications were excluded from this analysis. The Mantel-Cox log-rank method was used to test for differences between sex and translocation groups.

Distance to Human-Modified Areas by Season

We considered the effect of season on the distance that the centroid of the activity range was from SHM areas. For each snake, we calculated activity range centroids (mean of x and y coordinates) for each season, and averaged the corresponding seasonal values across years for those snakes tracked for parts of two or more years. We employed two ANOVA models: a one-way model considering season as a within-subjects factor, and the other a 2 x 3 x 4 (sex × translocation group × season) model including sex and translocation group as between-subjects factors, and season as a within-subjects model. Centroid distances to SHM areas were rank-transformed, and snakes that were not tracked for all four seasons were excluded from analyses. We included in our analysis only those snakes with at least one fix <50 m from an SHM area. We expected only those snakes familiar with SHM areas, and the resources they may contain, to deliberately move toward such areas.

Risk of Human Conflict and Return to Capture Site after Translocation

We utilized Cox proportional hazards models (Cox 1972) to address two questions related to the potential for conflict with humans: (1) What factors affect whether a snake moves near any area of potential human conflict? And (2) if a snake is translocated away from human conflict, what factors affect whether or not it returns to the place it was originally captured?

To assess the first question, we used a model that included snake sex and translocation group as factors, and the distance from each snake's initial release point to the nearest SHM area as a covariate. The time period used as the dependent variable began when the snake was released subsequent to transmitter implantation, and ended the first time that a snake moved within 50 m of any SHM area, or upon the last fix obtained for the snake, whether at the end of the study, when found dead in the field, or when found ill in the field and subsequently taken into captivity. For snakes lost due to a presumed transmitter failure, the final date was calculated as the midpoint between the last fix and the subsequent date when looked for but not found (Miller & Johnson 1978).

To assess the second question, our model looked again at sex and translocation group as fixed factors; however, this time we used initial translocation distance as the covariate. The time period used began when the snake was released after translocation and ended when the snake returned within 50 m of the point it was translocated from or to the final location fix for that snake in the study, as defined above. Only snakes that underwent at least one SDT from an area <50 m from an SHM area were used in this analysis, and only the first such event was considered to prevent pseudoreplication. This meant the initial translocation for all snakes in the SDT group was included. Snakes in the NT and LDT groups were included if, subsequent to initial release, they moved into SHM areas and underwent a follow-up SDT. In such cases only the first follow-up translocation events were included. We did not include the initial LDTs of those snakes in the LDT group in this analysis because, for several snakes in this group, the position of SHM areas (i.e. residential development) blocked, or would have significantly impeded, their return to their original capture location in large part because our protocol required that snakes that moved into such areas be translocated back towards areas were they been observed previously.

Since Cox proportional hazard models have few underlying assumptions (Mathew et al. 1999), no transformations were applied to the data. We confirmed the assumption of proportional hazard by checking log-log survival plots for parallelism between survival curves for each group.

Follow-up Translocations

Follow-up translocations subsequent to initial assignment of snakes to a translocation group (NT, SDT, LDT) were unavoidable given the legal issues that impacted our study design. This issue confounded our study design to some extent, particularly the distinction between NT and SDT snakes. To assess whether the number of follow-up translocations differed among the original translocation group assignments, we calculated the number of follow-up translocations per season and per year for each snake, and then data for seasons were averaged across years for each snake tracked during parts of two or more years. These values were then rank-transformed for the following analyses. We used a 2 x 3 factorial ANOVA model that used translocation treatment group and sex as independent variables. Because we did not detect a relationship between initially assigned translocation group and number of follow-up translocations (see Results), we did not control for follow-up translocations in the preceding analyses. To assess seasonal variation in the number of follow-up translocations, we used a two-way ANOVA model with season as a within-subjects factor and sex as a between-subjects factor. Snakes that were not follow-up translocated were excluded from this analysis.

Results

A total of 30 adult *C. ruber* provided telemetry data at various periods of time between the middle of 2008 and the end of 2011. Two of these snakes (snakes 2 and 11) were originally tracked as SDT snakes, but were then translocated into the western portion of the study site and placed in the LDT group, and treated separately for analyses (becoming snakes 2.1 and 11.1). This brought the total sample of snakes for analyses to 32 (Table 2). Median number of days tracked for these snakes was 387.5 days (range: 11–1148), and a location fix was obtained for each of these snakes on average (± SD) every 5.6 ± 3.1 days. Of these snakes, 10 were classed as NT snakes (4 males, 6 females), 12 were classed as SDT snakes (7 males, 5 females), and 10 were classed as LDT snakes (7 males, 3 females).

Activity Ranges and Movements

Depending on measure (MCP, LCH, RL), mean activity ranges averaged 38.6–67.1% larger for male snakes than those of female snakes, and LDT snakes had 20.0–56.3% larger activity ranges than SDT snakes, which had 77.0–152.9% larger activity ranges than NT snakes (Table 3, Figure 2). Results of the ANOVA models (Table 4) confirmed this trend; for MCP and LCH, the effects of sex ($P = 0.042$ and 0.015, respectively) and translocation group ($P = 0.004$ and 0.029, respectively) were both significant. Post hoc Tukey tests for both MCP and LCH showed that LDT > SDT = NT. For range length, only translocation group was significant ($P = 0.003$), with Tukey tests indicating LDT = SDT > NT; however, the effect of sex approached significance and had a large effect size ($P = 0.051$, partial $\eta^2 = 0.17$), suggesting that the range length of males was larger. No significant effects were detected for mean daily movement; however, the large effect size for translocation group (partial $\eta^2 = 0.19$; Table 4) suggested that

Table 2: Study dates, duration, and fate of radio–tracked Red Diamond Rattlesnakes (*Crotalus ruber*) near Loma Linda, California. Group indicates the translocation group of the snake: whether the snake was translocated (moved to a new location after transmitter implantation) a short distance (<710m, LDT) or a long distance (>710 m, SDT), or not translocated (NT). First date and last date indicate the first and last time telemetry data was collected for each snake.

I.D.	Sex	Group	First Date	Last Date	Days Tracked	Total Fixes	Fate
2	♀	SDT	8/2/2008	6/10/2009	313	62	Translocated to become snake 2.1
2.1[a]	♀	LDT	10/14/2009	10/21/2009	14	5	Died – killed by human action
3	♀	SDT	8/3/2008	10/12/2011	1145	180	Transmitter removed, snake released
4	♀	NT	8/10/2008	8/23/2009	384	68	Died – found depredated
5	♂	SDT	8/27/2008	2/16/2010	539	92	Died – surgery complications
6	♂	SDT	8/27/2008	10/12/2011	1121	151	Transmitter removed, snake released
7	♀	SDT	9/1/2008	9/15/2009	382	63	Lost – suspected transmitter failure
10	♂	SDT	9/16/2008	12/8/2008	88	3	Died – injury/surgery complications
11	♂	SDT	10/9/2008	10/14/2009	350	52	Translocated to become snake 11.1
11.1[a]	♂	LDT	10/21/2009	10/18/2011	721	90	Transmitter removed, snake released
12	♀	SDT	10/9/2008	5/7/2009	212	34	Lost – suspected transmitter failure
13	♂	SDT	10/21/2008	11/21/2008	36	6	Lost – suspected transmitter failure
14	♂	SDT	1/11/2009	1/27/2009	26	3	Died – suspected depredation
15	♀	SDT	1/26/2009	7/31/2009	193	31	Lost – suspected transmitter failure
16	♀	NT	3/13/2009	10/11/2011	929	165	Transmitter removed, snake released
17	♀	NT	3/19/2009	1/28/2010	215	42	Died – disease
18	♂	NT	3/19/2009	3/27/2009	11	2	Lost – suspected transmitter failure
19[b]	♀	LDT	10/14/2009	10/17/2011	706	131	Transmitter removed, snake released
20	♂	NT	10/21/2009	7/26/2011	631	122	Died – killed by human action
21	♀	LDT	4/7/2010	10/11/2011	559	80	Transmitter removed, snake released
22	♂	LDT	4/7/2010	4/12/2011	382	79	Died – unknown cause
23	♂	NT	4/9/2010	8/14/2011	424	78	Transmitter removed, snake released
24	♀	NT	5/13/2010	12/1/2010	204	45	Transmitter failed early; found alive 10/21/2013
25	♂	LDT	4/27/2010	10/11/2011	516	92	Lost – suspected transmitter failure
26	♂	NT	4/27/2010	12/30/2011	608	103	Not recaptured
27	♂	SDT	6/10/2010	12/30/2011	550	83	Not recaptured
28	♂	LDT	6/10/2010	10/18/2011	480	46	Transmitter removed, snake released
29	♀	NT	7/6/2010	6/15/2011	350	65	Lost – suspected transmitter failure
30	♀	NT	7/27/2010	8/23/2011	391	68	Transmitter removed, snake released
31	♂	LDT	8/5/2010	9/27/2011	423	62	Lost – suspected transmitter failure
32	♂	LDT	9/29/2010	9/19/2011	357	53	Lost – suspected killed by human action
33	♂	LDT	11/3/2010	10/24/2011	356	54	Transmitter removed, snake released

[a]Indicates an LDT snake that was tracked previously as a SDT snake.
[b]This snake was initially translocated 519 m; however, erratic movement patterns suggested she had been translocated outside of her normal home range so we assigned her to the LDT group.

LDT snakes exhibited more extensive movements than those of the SDT and NT groups (Table 3).

Table 3. Mean (± S.E.) activity range estimates (100% minimum convex polygon, MCP; 100% adaptive local convex hull, LCH; range length; RL) and movement descriptors (autocorrelation; t2/r2; mean daily movement; MDM) by translocation treatment (not translocated; NT; short distance translocated; SDT; long distance translocated; LDT) and sex.

Group	*N*	MCP (ha)	LCH (ha)	RL (m)	MDM (m)
Female					
NT	6	1.21 (±0.24)	0.73 (±0.18)	180.08 (±19.76)	7.09 (±1.09)
SDT	5	2.17 (±0.37)	0.90 (±0.17)	302.03 (±42.35)	6.04 (±1.00)
LDT	2	4.71 (±0.30)	1.71 (±0.16)	411.57 (±21.17)	9.82 (±1.23)
Male					
NT	3	2.60 (±0.35)	1.84 (±0.86)	260.86 (±7.99)	9.87 (±2.05)
SDT	4	3.88 (±1.05)	2.09 (±0.39)	371.94 (±61.30)	8.28 (±1.69)
LDT	7	7.17 (±1.30)	3.36 (±0.62)	476.17 (±58.24)	13.39 (±2.52)

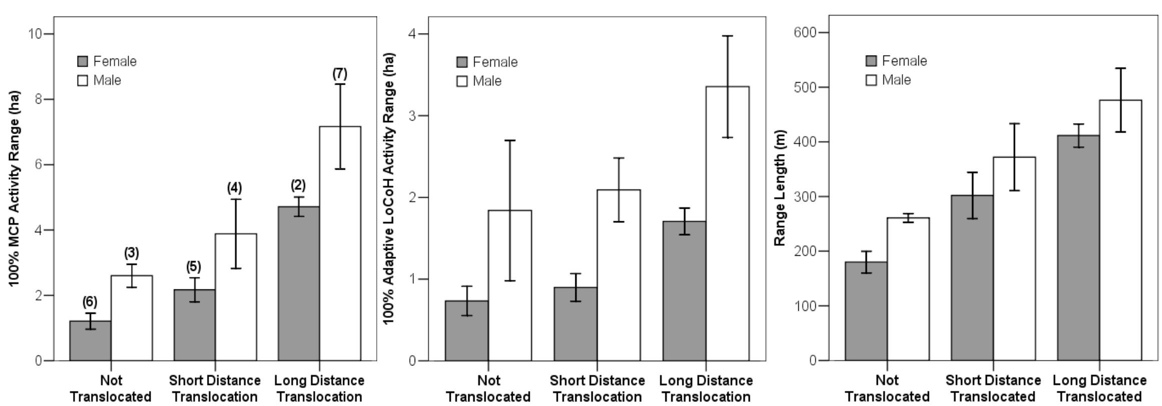

Figure 2. Mean activity range statistics (± SE) for long distance translocated (>716 m), initially short distance translocated (<716 m), and initially untranslocated Red Diamond Rattlesnakes (*Crotalus ruber*) of each sex. 100% minimum convex polygon (MCP; left), 100% adaptive local convex hull (LCH; center), and range length (right) are shown. Numbers in parentheses above the SE bars indicate the sample size for each group.

Table 4: Summary of analysis of variance (ANOVA) results comparing rank transformed estimates of home range (100% minimum convex polygon, MCP; 100% adaptive local convex hull, LCH), range length (RL), mean daily movements (MDM), and autocorrelation (t^2/r^2) between translocation treatment groups (no translocation, short-distance translocation, and long-distance translocation) and sex in Red Diamond Rattlesnakes (*Crotalus ruber*) in Loma Linda, California.

Dependent Variable	**Sex**			**Translocation**			**Interaction**		
	$F_{1,21}$	P	η^2	$F_{2,21}$	P	η^2	$F_{2,21}$	P	η^2
MCP	4.71	0.042*	0.18	7.23	0.004*	0.41	0.77	0.476	0.07
LCH	7.05	0.015*	0.25	4.22	0.029*	0.29	0.43	0.658	0.04
RL	4.27	0.051	0.17	7.83	0.003*	0.43	0.42	0.663	0.04
MDM	2.94	0.101	0.12	2.38	0.117	0.19	0.12	0.886	0.01
t^2/r^2	0.53	0.477	0.02	1.71	0.205	0.14	1.93	0.170	0.16

*$P < 0.05$
Effect sizes provided as partial η^2, and can be loosely interpreted as proportion of variance explained by each effect or interaction

Mean daily movement varied significantly by both season ($F_{3,69} = 18.57$, $P < 0.001$, partial $\eta^2 = 0.45$) and sex ($F_{1,23} = 152.68$, $P = 0.024$, partial $\eta^2 = 0.20$), and had large effect sizes. Pairwise least significant difference (LSD) tests revealed that snakes moved significantly less during winter compared to all other seasons ($P < 0.001$ for all), and that movements during spring, summer, and fall were similar ($P \geq 0.422$ for all). Median male movements were 16.7% greater than that of females. The interaction between season and sex was nearly significant, with a moderate effect size ($F_{3,69} = 2.65$, $P = 0.056$, partial $\eta^2 = 0.10$). An interaction plot (Figure 3) suggested that males moved more than females during the spring mating season.

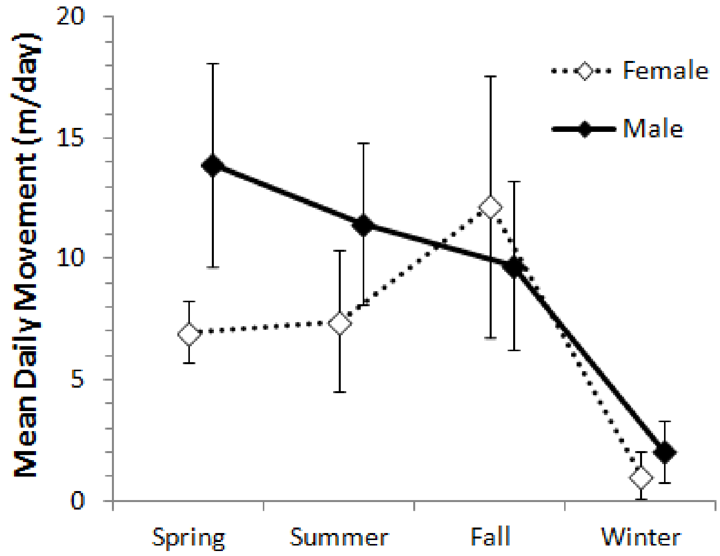

Figure 3. An interaction plot showing mean daily movement by season for male and female Red Diamond Rattlesnakes (*Crotalus ruber*).

Survival

Based on available evidence, we determined that nine deaths occurred during the study, two of which were related to complications from surgery. One of these two (snake 5) died during transmitter replacement surgery. The other (snake 10) was tracked for 88 days, but then sustained an injury that reopened the surgical incision and caused tearing, exposing a large portion of the coelomic cavity. The snake died four days later in our laboratory despite our best efforts to disinfect and close the wound. Of the six remaining deaths, two were likely the result of predation. We found the transmitter of snake 4 on open ground with unambiguous bite marks, and near a part of the snake's rattle containing the painted segments used for identification. We found the transmitter of snake 14 on the ground with probable bite marks, and without snake remains nearby. Two deaths resulted from the action of humans. We found the decapitated body of snake

2.1 in a plowed firebreak area 30 m from a home and a tennis court. The clean nature of the decapitation wound and absence of injury to the remaining body suggested a sharp object was used followed by burial of the head (which was not found). Snake 20 was buried by heavy earthmoving equipment as part of a landscaping project to expand the cemetery in the western portion of the study site. We confirmed this death by digging up the snake's remains. A third presumed case of human-caused mortality resulted when snake 32 moved into a large pile of brush and rocks within the area of the cemetery landscaping project. The pile was subsequently loaded by heavy equipment into large dumpsters and hauled away, presumably taking the snake away as well. In the remaining two cases of death, one resulted from apparent disease and the other was from unknown causes. We found snake 17 writhing and biting the ground in an area of coastal sage scrub, and subsequently euthanized the snake when it deteriorated further in our lab. We found the transmitter of snake 22 devoid of marks and in a large brush pile 24 m from a house.

We conducted two Kaplan-Meier analyses classifying each of the snakes above as ending in a mortality event. For the first test comparing the three translocation groups, the mean (± SE) survival time for all snakes was 853 ± 94 days, with 652 ± 110 days for NT snakes, 1033 ± 106 days for SDT snakes, and 575 ± 77 days for LDT snakes. A Mantel-Cox test showed no statistical difference between the three translocation groups (χ^2 = 0.77, P = 0.68). A separate test comparing males and females also found no significant difference (χ^2 = 0.01, P = 0.91). Mean survival time was 796 ± 131 days for males and 894 ± 126 days for females.

We conducted two more Kaplan-Meier analyses similar to the first one except that we excluded snakes that died due to human action. In the first model comparing translocation groups, the mean (± SE) survival time for all snakes was 973 ± 77 days, with 734 ± 116 days for NT snakes, 1033 ± 106 days for SDT snakes, and 677 ± 46 days for LDT snakes. Again, the Mantel-Cox test showed no statistical difference between the three translocation groups (χ^2 = 0.63, P = 0.730). A separate test comparing males and females again found no significant difference (χ^2 = 0.01, P = 0.923). Mean survival time was 961 ± 103 days for males and 962 ± 116 days for females.

To supplement the Kaplan-Meier analyses, we also compared first-year survival (≤365 days of tracking) of snakes in each group, excluding snakes whose eventual death involved surgical complications during the first year (snakes 5, 10). We ran two Chi-square analyses of survival for the three translocation groups, one excluding and the other including cases where deaths were attributable to human action. Results are shown in Table 5. Most snakes lost during the study without confirmed mortality resulted from transmitter failure; thus, mortality could not be determined for these individuals. Omitting these lost snakes, there were no differences between groups when human-caused mortalities were excluded (N = 23, df = 2, χ^2 = 1.18, P = 0.553, Cramer's V = 0.23) or when they were included (N = 24, df = 2, χ^2 = 0.036, P = 0.982, Cramer's V = 0.04). Thus, first-year survival rates were comparable for non-translocated (85.7% of N = 10 snakes) and translocated snakes (both groups combined: 80.0% of 20 snakes excluding human-caused mortality, 80.9% of 21 snakes including human-caused mortality), with small effect sizes.

Table 5. Summary of first-year survival (≤ 365 days of tracking) for radiotransmittered snakes in each translocation group (NT, not translocated; SDT, short-distance translocation; LDT, long-distance translocation). Survival rate excludes snakes lost during the study, presumably from transmitter failure. One snake mortality due to surgical complications that occurred during the first year of tracking is omitted.

Group	N	Survived (%)	Lost (%)	Confirmed dead (%)	Survival rate (%)
Without Human Mortality					
NT	10	60.0	30.0	10.0	85.7
SDT	11	63.6	27.3	9.1	87.5
LDT	9	89.0	11.1	0.0	100.0
With Human Mortality					
NT	10	60.0	30.0	10.0	85.7
SDT	11	63.6	27.3	9.1	87.5
LDT	10	80.0	10.0	10.0	88.9

Distance to SHM Areas by Season

The average distance of snakes from SHM areas varied by season (Figure 4A). The one-way repeated-measures ANOVA showed a nearly significant effect of season and a moderate effect size (N = 19, $F_{3,54}$ = 2.52, P = 0.068, η^2 = 0.12). The mixed factorial ANOVA including season, sex, and translocation group similarly showed season to approach significance with a fairly large effect size (N = 19, $F_{3,39}$ = 2.32, P = 0.090, partial η^2 = 0.15). Pairwise comparisons using the least significant difference (LSD) method showed significant differences, with distance to SHM areas being less in summer than in spring or winter (P = 0.032 and 0.048 respectively; Figure 4A). Translocation group ($F_{2,13}$ = 0.14, P = 0.872, partial η^2 = 0.02) and sex ($F_{1,13}$ = 0.15, P = 0.707, partial η^2 = 0.01) were not significant, and no significant interactions were detected.

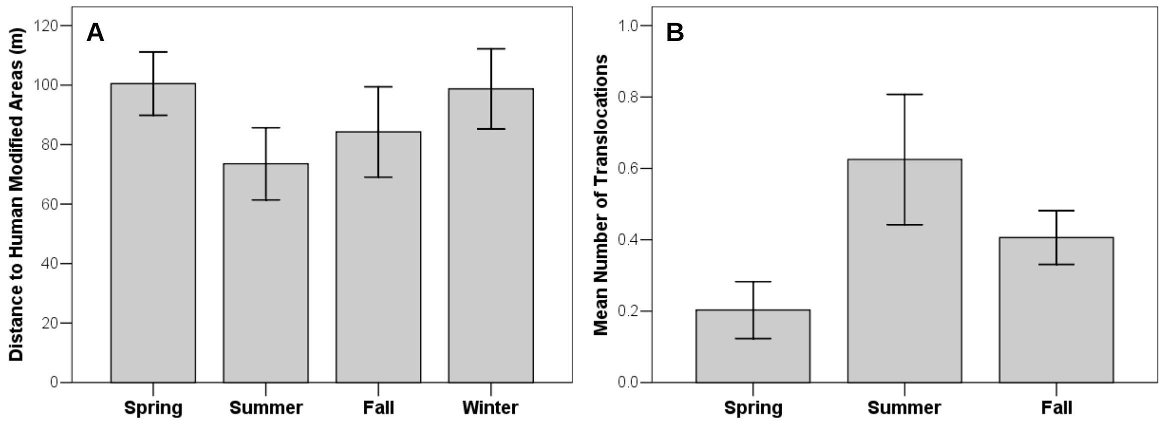

Figure 4. (A) Mean (± SE) distance to areas of significant human modification for 19 snakes across four seasons. (B) Mean (± SE) number of follow-up translocations for 16 snakes across three seasons. Winter is omitted because no follow-up translocations from areas less than 50 m from areas of significant human modification occurred during this season.

Risk of Return to Human-modified Areas

Following transmitter implantation, snakes (including those from all three translocation groups) were initially released an average of 152.5 m (range: 25.9–459.5 m) away from the nearest SHM area. Omitting two snakes initially released <50 m away from SHM areas, 73.3% of all snakes (22 out of 30), regardless of translocation group, moved to areas within 50 m of SHM areas subsequent to initial release. Median time for these snakes to move to these areas was 48 days (95% CI = 0.00–141.18) based on Kaplan-Meier analysis. Cox regression revealed that an increase in distance between a snake's release point and SHM areas reduced the risk of a snake moving near such areas by a small but significant amount (hazard ratio [HR] = 0.988, 95% CI = 0.977–0.99, N = 30, df = 1, Wald = 4.83, P = 0.028). The model suggested that, holding other factors constant, for every 1 m increase in distance from the release point to SHM areas, the risk of returning to such areas decreased by 1.2% (95% CI = 1.0–2.3%). Translocation group also affected the risk of a snake moving near SHM areas (*N* = 30, df = 2, Wald = 9.00, *P* = 0.011), with LDT (HR = 5.92, 95% CI = 1.39–25.21, Wald = 5.78, df = 1, *P* = 0.016) and SDT snakes (HR = 10.18, 95% CI = 2.23–46.45.21, Wald = 8.97, df = 1, *P* = 0.003) having a significantly greater risk of moving near SHM areas than NT snakes (Figure 5). Sex was not significant (Wald = 3.42, df = 1, *P* = 0.065).

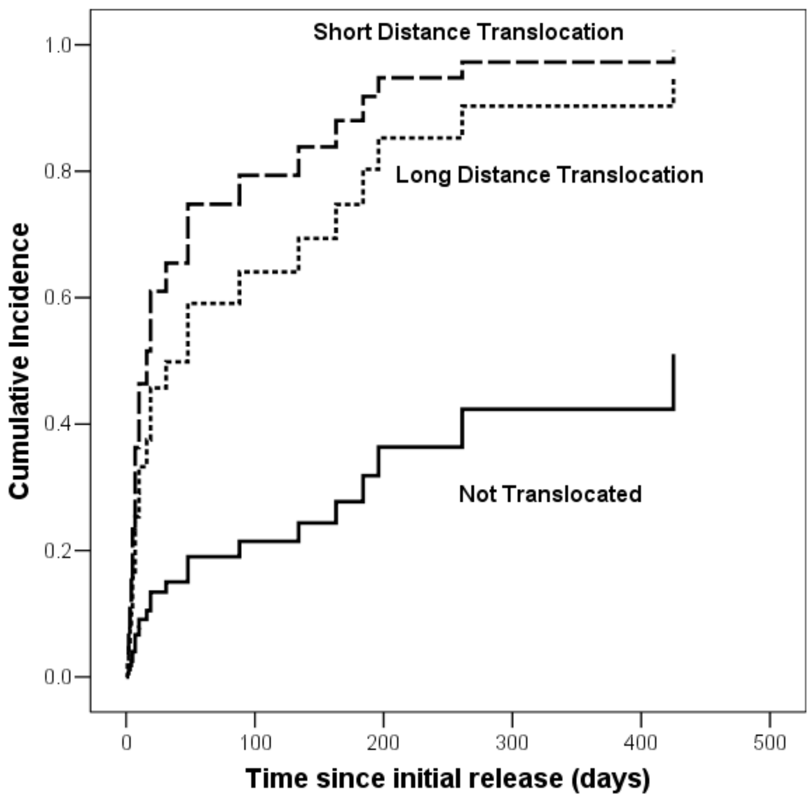

Figure 5. Cumulative incidence of snakes moving within 50 m of an area of significant human modification after initial release. A separate curve is shown for each translocation group.

Risk of Return to Area of Capture after Translocation

Of the 22 snakes that were short-distance translocated away from SHM areas at some point during the course of the study, the first such translocation for these snakes averaged 246.2 ± 155.7 m (range 32.2–633 m). Omitting one snake that was translocated less than 50 m, 52.4% of these snakes returned to within 50 m of their original location. Median time to return was 163 days (95% CI = 11.1–314.9) based on Kaplan-Meier analysis. Cox regression again showed a small but significant effect of the distance the snake was translocated away from its capture site (N = 21, HR = 0.985, 95% CI = 0.937–0.998, Wald = 5.07, df = 1, P = 0.024). This model suggested that, keeping other variables constant, for every 1 m increase in the translocated distance, the risk of return decreased by 1.5% (95% CI = 0.2– 6.3%). Sex (Wald = 1.88, df = 1, P = 0.170) and translocation group (Wald = 0.34, df = 2, P = 0.842) were not significant. None of the LDT snakes returned to within 50 m of their initial capture sites except for the one female (snake 19) which was initially translocated 519 m but was assigned to the LDT group due to extensive and erratic movements after translocation. Another LDT snake (snake 33), translocated 2573 m from his initial capture site, appeared to make an effort to return. Subsequent to initial release on 1 November 2010, this snake moved 681 m in a direction toward its capture site until 11 November 2010, when it moved into a residential area and on the grounds of an elementary school. It was then translocated 633 m back to its original translocated position. The snake again moved 618 m in a direction towards its capture site and returned to within 126 m of the same elementary school on 15 December 2010. At this point, to prevent the snake from returning to the elementary school, we broke protocol and again recaptured the snake prior to re-entering an area of potential human conflict (which is why this translocation was excluded from the translocation analysis above). We translocated this snake 582 m (straight-line distance) back to the point of its initial release. It then overwintered near this release point. The snake did not move in the direction of its capture location the following spring, and was found paired with a female on 3 March 2011.

Follow-up Translocations

Sixteen snakes underwent short-distance follow-up translocation at least once during the study, including 4 of the 10 NT snakes (40.0%), 6 of the 12 SDT snakes (50.0%), and 6 of the 10 LDT snakes (60.0%). For these snakes, we conducted an average of 3.6 (SD = 3.1) translocations per snake per year. The number of follow-up translocations per season per year was independent of assigned treatment group ($F_{2,21}$ = 1.35, P = 0.28, η^2 = 0.11) and sex ($F_{1,21}$ = 0.02, P = 0.89, η^2 = 0.001), and there was no significant interaction between sex and treatment group ($F_{2,21}$ = 2.05, P = 0.153, η^2 = 0.16).

The number of follow-up translocations varied by season (Figure 4B), with the highest frequency in summer and none occurring during winter. The one-way repeated-measures ANOVA for the three seasons showed a significant effect (N = 16, $F_{2,30}$ = 3.53, P = 0.042, η^2 = 0.19), with pairwise LSD comparisons suggesting significantly fewer follow-up translocations during spring compared to summer and fall (P = 0.045 and 0.020 respectively). The mixed factorial ANOVA including season and sex approached significance for season and showed a relatively large effect size (N = 16, $F_{2,28}$ = 3.24, P = 0.054, η^2 = 0.19). Sex ($F_{1,14}$ = 0.71, P = 0.42, partial η^2 = 0.05) was not significant.

Discussion

Mitigation translocation has received increasing attention as an option for dealing with nuisance wildlife, including venomous snakes. However, three key concerns have been raised: 1) the potential of harm to the translocated snake; 2) return of the snake to the original site of human-snake conflict; and 3) population-level effects that result from the translocated animal. In this study, we radiotracked three groups of adult rattlesnakes to improve our understanding of the first two concerns. If translocation represents a successful management tool for reducing human-snake conflict, it should minimally impact the translocated snake while reducing the likelihood of the snake returning to a site of potential conflict. We sought to learn how distance of nuisance snake translocation from human-modified areas would impact their subsequent spatial ecology, movements, survival, and, ultimately, their potential for conflict with humans. Because of property owner requests and liability issues at our study site, we were compelled to translocate all snakes that were initially found in human-modified areas, and those that returned subsequently to these areas. In spite of this complication to our study design, we were able to assess not only the role of translocation distance in translocation success, but also differences between the sexes and among the four seasons.

Translocation Effects on the Snake

One major concern about mitigation translocation is the potential for negative impacts on the snake. We found the activity ranges (both MCP and LCH) of LDT snakes to be 1.5 to 4.0-fold larger than those of SDT and NT snakes. Range lengths for both LDT and SDT snakes were also 1.9 to 2.2-fold larger than those of NT snakes. Translocation group differences, however, were less evident from mean daily movements, though the relatively large effect size was consistent with the trends for activity ranges and range length. These results are consistent with those of most studies of rattlesnakes to date, which also have found that translocated snakes exhibit larger activity ranges and move greater distances than non-translocated snakes, at least in the first year of study (Table 1).

Males in our study also exhibited 1.4 to 1.7-fold larger activity ranges and 1.2-fold greater daily movements than females, which is typical of *C. ruber* (Brown et al. 2008) and other rattlesnakes (e.g. D. Duvall & Schuett, 1997; Jellen, Shepard, Dreslik, & Phillips, 2007; Glaudas & Rodríguez-Robles, 2011). We found no interaction between sex and translocation group in home range size and movements, suggesting that both sexes responded similarly to the translocation. However, we did find a nearly significant interaction between season and sex that may suggest that males move greater distances than females in the spring. This is unsurprising, as spring is the mating season for *C. ruber* (Dugan et al. 2008). Rattlesnakes, as with pitvipers generally, exhibit prolonged mate searching polygyny in which males search competitively for widely distributed, spatially unpredictable, and/or scarce females (Duvall et al. 1992). In the process of searching for females, males traverse long distances, potentially in previously unexplored areas, and, consequently, garner larger home ranges (Aldridge & Duvall 2002). Accordingly, sex-based differences may exist in the behavior of snakes in unfamiliar surroundings and, ultimately, in their tolerance to LDT. Because males roam widely when searching for females, they may be equipped with behavioral and physiological adaptations that enable them to navigate and otherwise cope with being in previously unexplored areas, which females, who move much less and have smaller home ranges

(Aldridge & Duvall 2002), may lack. The presence of such physiological adaptations has been suggested by the findings of Holding et al. (2012, 2014) and Heiken (2013). Although only male *C. oreganus* was used in both studies, snakes repeatedly subject to SDT developed larger medial cortexes than controls, presumably in response to increased navigational demands (Holding et al. (2012, 2014), and increased testosterone levels in LDT snakes may aid in spatial learning and memory (Heiken (2013). Both studies looked at levels of the stress hormone corticosterone and found no difference between translocated and control snakes. If males are adapted to cope with unfamiliar areas, then such a reduced stress response would be expected.

Although our snakes exhibited increases in space use and movements corresponding to distance of translocation, survival (overall and during the first year) was similar among the three translocation groups and between sexes. Moreover, survival in the first year was similar among groups and between sexes regardless of whether we included (87.5% for all snakes) or excluded (91.3% for all snakes) human-caused mortalities. Thus, at our study site, translocation of nuisance snakes neither subjected them to nor protected them from higher levels of mortality. Effect sizes (Cramer's *V*) comparing survival between translocation groups of snakes were in the range of 0.04 to 0.23, depending on whether human-caused mortalities were included, which we interpret as small to moderate (Cohen, 1988). With much larger sample sizes, the differences could certainly become significant, but effect size has more biological relevance than does significance (Nakagawa & Cuthill 2007).

Our results were generally consistent with those of previous studies, whose effect sizes (Cramer's *V*) for longer-term studies ranged from 0.19 to 0.47 (relatively small to large; Table 1). Prior research has shown no differences in mortality for SDT snakes compared with controls (Brown et al. 2009; Holding et al. 2012; Holding 2011; Holding et al. 2014; Brown et al. 2008). For LDT snakes, however, previous studies offer mixed conclusions. Brown et al. (2008), who also examined *C. ruber*, did not detect a difference in mortality between LDT and non-translocated snakes, but other studies of *C. atrox* and *C. horridus* reported that LDT snakes suffered higher mortality (Reinert & Rupert, 1999; Nowak et al., 2002; Heiken [2013] observed no mortality in both control and LDT snakes, but the study duration was only one month). The difference between *C. ruber* and other species may be due to differences in habitat and/or dependence upon specific hibernacula for overwinter survival. In cooler climates, snakes unable to locate suitable locations to brumate (≈ hibernate) are much less likely to survive the winter (Nowak & Riper 1999). Indeed, a significant number of mortalities among LDT snakes in Reinert & Rupert's (1999) study occurred during the winter, and high winter mortality has been reported in repatriated Massasuaga rattlesnakes (*Sistrurus catanatus*; King et al. 2004; Harvy & Lentini 2013). In warmer climates, such as southern California, the need to utilize specific or communal hibernacula to escape freezing conditions is reduced (Dugan et al. 2008), and hence the inability to find ideal hibernacula may have less of an effect on survival. We encourage future researchers to emphasize effect sizes when comparing the behavior and survival of translocated snakes to control snakes. Because these studies involve radiotelemetry and intense tracking effort, sample sizes are generally small, which hampers statistical power. Effect sizes are independent of sample size and can be more readily compared among different studies (Nakagawa & Cuthill 2007).

Risks Associated with Human-Snake Conflict

The second major concern of mitigation translocation is whether the snake is less likely to return to the area of potential human-snake conflict. We considered three measures of potential conflict in relation to snake translocation: 1) average distance of the snake from human-modified areas; and 2) whether snakes returned to human-modified areas. Prior studies of snake translocation have only considered the latter measure. In terms of distance from human-modified areas, seasonality appeared to have the strongest influence. Our snakes, regardless of sex or translocation group, moved closer to SHM areas and were subject to a greater number of follow-up translocations in summer (and to a lesser extent in fall) than during spring or winter (Figure 4B). This seasonal effect contrasts with the seasonal occurrence of snake envenomations in southern California, which peak in the spring (Parrish et al. 1964; Wingert & Chan 1988; Chapter 4 this dissertation). Although a significant proportion of snake envenomations occur at the victim's home (Parrish et al. 1964; Tokish et al. 2001; Minton 1987), human envenomations appear to be associated more strongly with seasonal increases in snake movement. Indeed, *C. ruber* shows highest levels of activity during the mating season in spring (T. K. Brown et al., 2008; Dugan et al., 2008; present study), when most human envenomations occur. Of course, a snake isn't a nuisance unless it is discovered (Sealy 1997; Sealy 2002), and snakes are presumably more likely to be encountered or detected by humans when they are moving. Indeed, one study reported that human infants showed a differential response to snake images over that of other animals only when shown a video of a moving snake (DeLoache & LoBue 2009).

The number of snakes requiring follow-up translocation because they returned to human-modified areas was most frequent during summer, regardless of sex (Figure 4B). A high proportion (73.3% of 30) of snakes moved near SHM areas at some point during the study (median of 48 d after initial release), and roughly half of those translocated a short distance away from SHM areas (52.4% of 22) returned to within 50 m of their original capture site in an SHM area (median of 163 days). Translocated snakes of both groups were 5.9 to 10.2-fold more likely to move near SHM areas than were NT snakes, regardless of sex. The difference between groups is not surprising, since NT snakes tended to have established home ranges that were more distant to SHM areas, whereas translocated snakes were classified as such because their home range brought them to SHM areas. However, the risk of a snake moving to a SHM area decreased at approximately 1.2% per 1 m distance of initial release from a SHM area. For those snakes moved a short-distance at some time during the tracking, the probability of returning to close proximity (within 50 m) of their initial capture site was independent of translocation group and sex. Some snakes in all groups were subjected to this translocation, so the lack of differences between translocation groups seems expected. But more important, the risk of a snake moving to a SHM area decreased at approximately 1.5% per 1 m distance of release from a SHM area.

Snakes subjected to LDT may nevertheless experience negative impacts. Such individuals may take time to orient themselves to the new environment and, as a result, increase their movements and exposure to risks as they search for suitable areas to forage, bask, shelter, and locate mates. If these snakes exhibit natal habitat preference induction and have established natal home ranges that include SHM areas, then such snakes may seek such areas once translocated, and either come into conflict with humans again, or move into sub-optimal habitats that the snake perceives to be similar to SHM areas, and thereby increase their risk of mortality (Stamps & Swaisgood 2007). In our study, the risk of LDT snakes moving near SHM areas was about six fold greater than that of NT snakes, and equivalent to SDT snakes. This may be explained in at least three ways. First, LDT snakes may have been attempting to navigate back to the area near their point of capture, and SHM areas existed between their position and their intended destination. This seemed to be the case for snake 33, whose movements suggested deliberate navigation toward its capture site. Its movement into an SHM area was likely because such areas just happened to be on his intended path. Second, LDT movements into SHM areas could have been facilitated by the larger home-ranges and increased movement patterns exhibited by these individuals. Third, LDT snakes may actively seek SHM areas subsequent to translocation because they have developed a preference for such areas due to prior foraging (or mating) success, or even natal habitat preference induction (Stamps & Swaisgood 2007). Though little evidence exists of natal habitat preferences in reptiles (Germano & Bishop 2009), it has been documented in a wide range of taxa, including amphibians, birds, and mammals (Davis & Stamps 2004), so it also likely exists in reptiles as well.

The Effect of Follow-up Translocations

The obligation from legal counsel to conduct follow-up short-distance translocations created the potential that these extra translocations would confound the effect of our initial translocation treatment groups. However, several lines of evidence suggest that our comparisons of the three treatment groups remain largely valid. First, only the LDT snakes were subjected to a (single) long-distance translocation, which maintained this group's independence from the others. Second, only 50% of the NT snakes experienced SDT in the form of follow-up translocation, whereas all of the SDT snakes experienced SDT. Although the follow-up translocations likely reduced the magnitude of response differences between these two groups, some differences still emerged. Third, the mean number of follow-up translocations per season per year was statistically similar for the three treatment groups, suggesting an equivalent effect of this treatment for all groups.

Implications for Managing Nuisance Rattlesnakes

Those who deal with human-wildlife conflict must take into consideration three major issues when mitigating situations involving nuisance animals. Our research has addressed the first two issues for rattlesnakes, which include the potential negative impact to the individual nuisance animal, and the risk to humans (or property) posed by the animal. The third issue is the potential negative impact to the population the animal is a part of and/or the population it may be moved to if translocated. We will comment on each of these as they relate to managing nuisance rattlesnakes.

With regard to welfare of the nuisance animal, there are three major options for dealing with nuisance rattlesnakes: (1) leaving the snake alone, which a property owner often objects to; (2) euthanasia, which has the most deleterious impact to the snake, and is therefore considered least humane; or (3) removal of the live snake, either by translocating it to another area or maintaining it indefinitely in captivity. For translocation, a trade-off exists between immediate effects on the snake's behavior, including an increased risk of death (the major problem with LDT), versus future return to the area of conflict, which could result once again in human conflict and death of the snake (the major problem with SDT). Heretofore, LDT has been strongly criticized as a strategy that unacceptably increases the probability of snake death (Reinert & Rupert 1999; Nowak et al. 2002; Sullivan et al. 2014). However, two studies of *C. ruber* (ours and Brown et al. 2008) suggest that LDT can be a viable option for at least some species, or for snakes in certain climates or habitats. Much of the mortality reported for translocated snakes of other species has been associated with brumation ≈ hibernation (Reinert & Rupert 1999; Harvey et al. 2014), often at communal hibernacula. Repatriated snakes (captive-raised prior to release in the wild) have similarly been especially vulnerable during the hibernation period (King et al. 2004). The milder climate of southern California, where many snakes overwinter individually without strong site fidelity (Dugan et al. 2008), may reduce the risk of overwinter mortality in translocated snakes. Other factors no doubt contribute to translocation success, including the species' biology (*C. ruber* is a relatively sedentary species; Dugan et al. 2008), snake population density, and availability of prey and refugia, At present, we find no compelling evidence to recommend against LDT in the southern California region, except at higher altitudes where communal overwintering at scarcely-distributed suitable sites may be critical for survival. Nevertheless, we certainly agree with Sullivan et al. (2014) that public education about tolerating nuisance rattlesnakes would be a viable alternative or supplement to mitigation translocation.

Although LDT may increase the probability of rattlesnake mortality in the short term, at least the snakes are given a chance to succeed. Some consider the reduced survival rate of LDT (as low as 45.5%; Table 1) unacceptable (Reinert & Rupert 1999;

Sullivan et al. 2014); however, all wild snakes will experience death eventually through illness, starvation, depredation, and/or senescence, any of which might cause equal levels of suffering. Many LDT snakes live beyond the first year of highest vulnerability, and thereafter may thrive as successfully as non-translocated snakes (Reinert & Rupert 1999). In the absence of studies showing substantially lower levels of survival (well under 50%), we see no reason why LDT would be more unethical than euthanasia, particularly in areas where SDT is not an option permitted by property owners.

With regard to risk of the nuisance animal to humans or property, venomous snakes actually pose a very low risk to humans in the United States. The most recent estimates put the incidence of snake envenoming at 0.79 per 100,000 per year, and the incidence of death from such envenomings at 0.001 per 100,000 per year (Kasturiratne et al. 2008). Certainly the risk is higher for those people who live in more rural areas, those who spend significant time in natural areas for recreational or occupational purposes, and those whose health may be somewhat compromised. Yet in many cases human envenomings occur because a human deliberately chooses to interact with the snake, either to harass, kill, or capture it (see Chapter 3), suggesting that the risk of envenomation would be significantly reduced if people would simply choose to leave the snakes alone. In spite of the fact that our snakes most frequently approached and entered SHM areas during the summer, the majority of human envenomations in our region occur during spring (Parrish et al. 1964; Wingert & Chan 1988; Chapter 3 this dissertation). This discrepancy suggests that simple proximity to SHM areas may be a poor predictor of the overall risk to humans posed by venomous snakes.

Although no research has examined the third issue for rattlesnakes, strategies for mitigating human-wildlife conflict may have effects at the population level (Burke 1991; Reinert 1991; Chipman et al. 2008; Massei et al. 2010; Sullivan et al. 2014). Certainly, indiscriminant use of euthanization has the potential to deplete local populations—an outcome that can be particularly undesirable if the species in question is protected, as in our study, or is a high trophic level consumer (i.e., predator), which can lead potentially to profound ecosystem change (Shine & Koenig 2001; Estes et al. 2011; Sullivan et al. 2014). Moreover, euthanization may alter sex ratios by effecting one sex more than the other. Previous studies indicate a male bias in human-caused snake mortality (Bonnet et al. 1999; Shepard et al. 2008), and male rattlesnakes may be responsible for more human envenomations than females (Cardwell et al. n.d.), suggesting they may be more likely to come into conflict with humans.

Translocations involving substantial distance have the potential to affect both the source population (assuming absence of homing to the original location) and the population the animal is moved to. In the case of rattlesnakes, individual cases of conventional LDT (beta-translocation) involve distances too limited to affect anything more than the immediate neighbors of the translocated snake. Given the dynamic nature of snake home ranges, which contract and expand seasonally and from year to year, and generally overlap widely the ranges of other snakes (Macartney et al. 1988), any impacts from the translocation of a few snakes should be fairly negligible. More problematic would be removal of an excess number of snakes from a given location, as the local population could suffer predator depletion. Sex ratios might also become skewed in both the source (female-biased) and receiving (male-biased) populations. These risks are not only dependent on the distance these snakes are moved, but also on the number of animals moved. Nuisance snake dumping, involving dozens of individuals at the same location, has been described in Arizona (Mccrystal & Ivanyi 2008), and no doubt has negative repercussions for the dumped snakes and the local environment. With regional translocation beyond the local deme (gamma-translocation), the translocated snake may become a vector of disease transmission to the new population, alter the genetic structure of the new population, or increase the receiving population beyond carrying capacity (Reinert 1991; Shine & Koenig 2001). For rattlesnakes, the consequences of gamma-translocation have not been examined, but there are compelling reasons to recommend against this practice unless it is part of a carefully monitored repatriation program (e.g. Harvey et al. 2014 and references therein).

Conclusions

This study investigated nuisance snake translocation as a management tool for reducing human-snake conflict. Although rattlesnakes translocated beyond their normal home range exhibited increased space use and movements, those moved greater distances from human-modified areas were less likely to return, and they experienced survival rates similar to those moved short distances. Thus, our findings add to the growing body of evidence that translocation of nuisance snakes can be a viable approach to reduce human-snake conflict, at least for some species and/or locations. In spite of accumulating studies on the effects of translocation on snakes, this form of mitigation remains a highly experimental approach for which generalizations should be made with caution. Studies vary substantially in their translocation protocols, duration, and assessments of behavior and mortality, and all are constrained by relatively small samples. Standardized terminology for translocation distances, such as the alpha-, beta-, gamma-, and delta-translocation rubric we introduce here, can reduce the confusion arising from different meanings of the terms SDT and LDT among different studies.

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CHAPTER THREE

FACTORS THAT INFLUENCE THE ETIOLOGY AND CLINICAL SEVERITY OF VENOMOUS SNAKEBITES IN SOUTHERN CALIFORNIA

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Abstract

We retrospectively reviewed 354 cases of venomous snakebite admitted to Loma Linda University Medical Center (LLUMC) between 1990 and 2010. Our aims were to assess the factors that influence the etiology and clinical severity of rattlesnake bites, and to evaluate the clinical effectiveness of current antivenom (CroFab®) in resolving symptoms caused by venoms of the seven medically significant snake taxa native to southern California. We assessed four measures of snakebite severity: initial snakebite severity scores (iSSS) prior to administration of antivenom and at maximum severity (mSSS), vials of antivenom administered, and duration of hospitalization. Of the cases reviewed, 80.5% were male, 69.2% of bites were to an upper limb, and 88.0% were distal to the wrist or ankle. Of 308 cases where a determination could be made, 45.8% were illegitimate (provoked bites), and 18.0% involved alcohol or illegal drugs. Most snakebites occurred during the spring mating season, followed by another large pulse during fall associated with newborn snake emergence. Snakebite severity was positively associated with snake size, negatively associated with patient mass, and independent of patient age, snake taxon, anatomical location of bite, legitimate versus illegitimate (provoked) bites, and time until hospital admission. Male snakebite victims were 2.9, 7.1, and 3.1 times more likely than female victims to sustain bites to the upper extremity, distal to the ankle or wrist, and via illegitimate provocation, respectively. Those admitting to alcohol or drug use were 5.7 times more likely to sustain illegitimate bites, which were 111.0- and 7.1-fold more likely than legitimate bites to be to the upper limb and distal to the ankle or wrist, respectively. Despite concerns that CroFab is ineffective in neutralizing the venom of some snake taxa, especially that of the Southern Pacific

Rattlesnake (*Crotalus oreganus helleri*), we found its clinical effectiveness to be similar for all taxa.

Introduction

Venomous snakebite represents an important and largely neglected global public health issue (Gutiérrez et al. 2006; Harrison et al. 2009; Simpson & Norris 2009). Recent estimates suggest that as many as 2.5 million people are envenomed by snakes every year, of which as many as 94,000 die (Kasturiratne et al. 2008; Gutiérrez et al. 2010). Although mortality is low in the United States (estimated 5–7 deaths annually; Kasturiratne et al. 2008), venomous snakebite is still a significant medical issue when it occurs. The several thousand people who are envenomated by snakes in the U.S. annually (Parrish 1966; Kasturiratne et al. 2008; Smith & Bush 2010) can develop potentially life-threatening hematotoxicity and neurotoxicity, as well as significant local soft tissue damage that can result in long-term physical and emotional morbidity (Dart et al. 1992; Smith & Bush 2010; Williams et al. 2011).

In the U.S., southern California suffers a relatively high rate of venomous snakebite. Between 2001 and 2005, Californians reported 1,420 (284 per year) venomous bites to the American Association of Poison Control Centers, which was the fourth highest of any U.S. state (Seifert et al. 2009). Most cases likely occur in the southern part of the state (Parrish et al. 1964). Human population growth and associated urban sprawl have spawned development of wilderness areas, bringing humans in conflict with local wildlife, including the seven taxa of rattlesnakes which comprise the only venomous snakes (apart from the rear-fanged colubrids) native to the area (Campbell & Lamar

2004; see Table 1). Bites in southern California may average more severe than those in other regions of the U.S., which have Cottonmouths and Copperheads (genus *Agkistrodon*) and Coral Snakes (genera *Micrurus* and *Micruroides*) in addition to the rattlesnakes (genera *Crotalus* and *Sistrurus*) that are more likely to cause major clinical effects or death (Langley 2008; Seifert et al. 2009).

Table 1. Bites caused by the seven taxa of southern California rattlesnakes in this study.

Species of Rattlesnake	Number of Bites	Percent of Total Bites
Southern Pacific (*Crotalus oreganus helleri*)	95	26.8
Sidewinder (*Crotalus cerastes*)	32	9.0
Mohave (*Crotalus scutulatus*)	28	7.9
Red Diamond (*Crotalus ruber ruber*)	21	5.9
Speckled (*Crotalus mitchellii pyrrhus*)	12	3.4
Western Diamond-backed (*Crotalus atrox*)	9	2.5
Northern Pacific (*Crotalus oreganus oreganus*)	7	2.0
Unknown	150	42.4

Two important issues relate to venomous snakebite: etiology and clinical severity. Understanding etiology informs methods of preventing bites, whereas understanding the factors that affect clinical severity informs treatment. When considering etiology, a distinction is often made between “illegitimate” and “legitimate” bites. Klauber (1956) defined legitimate bites as those “that happen to persons who have no intention of indulging in so unnecessary a risk,” whereas Russell (1983), taking a cue from Klauber, defined an illegitimate bite as one inflicted on “those who, by their own decision, chose to handle snakes, or otherwise expose themselves to risk.” Studies prior to the 1980s suggested that the majority of snakebites in the U.S. were legitimate, particularly to children (Hutchison 1929; Parrish 1966; Russell 1983). However, more recent studies have yielded somewhat contradictory conclusions. Several studies reported a majority of illegitimate bites (e.g. Wingert & Chan 1988; Curry et al. 1989; Morandi & Williams 1997; Janes et al. 2010), whereas other authors (e.g. Dart et al. 1992; Plowman et al. 1995) reported a majority of legitimate bites.

Similar inconsistencies exist in the literature on clinical severity. Several factors have been postulated to contribute to the clinical severity of a bite. These factors have been partitioned into those associated with the snake and those associated with the human victim (Hayes & Mackessy 2010). In terms of the snake, hospital-based studies have consistently shown that larger snakes tend to cause more severe bites (Wingert & Chan 1988; Hayes et al. 2005; Janes et al. 2010; see also Hackett et al. 2002) because they inject larger quantities of venom (Hayes 1991; Hayes 2008). However, the idea that more provoked snakes deliver more venom, and therefore a more severe bite, has had mixed support, especially for rattlesnakes (Herbert 1998; Rehling 2002). Venom composition, which varies with ontogeny and among populations and taxa, also affects clinical severity (Hayes & Mackessy 2010; Massey et al. 2012). In terms of the human victim, evidence supporting the suggestion that smaller patients tend to have more severe bites has also been mixed. While some studies have supported this contention (Hayes et al. 2005; Pinho et al. 2005), others have failed to detect this relationship (Parrish et al. 1965; Janes et al. 2010). Bite severity might also be influenced by site of the bite, dictated largely by human behavior (Wingert & Chan 1988; Moss et al. 1997; Tanen et al. 2001); presence of clothing (Herbert & Hayes 2009); general health (Tanen et al. 2001; Benítez et al.

2007; Ribeiro et al. 2008); delay to treatment (Pinho et al. 2005; Michael et al. 2011; Paul & Dasgupta 2012; Saravu et al. 2012); and the treatment itself.

Another issue of importance to clinicians treating venomous snakebites is the effectiveness of antivenom at neutralizing venom toxins and resolving symptoms. Some controversy surrounds the effectiveness of Crotalidae Polyvalent Immune Fab (Ovine) (CroFab®; Protherics, Brentwood, TN, USA, now part of BTG International, London, UK) in neutralizing the venom of the Southern Pacific Rattlesnake (*Crotalus oreganus helleri*), a taxon which exhibits substantial geographic variation in venom composition (French et al. 2004; Jurado et al. 2007; Salazar et al. 2009; Sunagar et al. 2014). CroFab is produced from hyperimmune sera derived from sheep inoculation with four snake venoms: *C. adamanteus*, *C. atrox*, *C. scutulatus*, and *Agkistrodon piscivorus*. Previous research suggested that CroFab was much less effective in neutralizing the venom of *C. o. helleri* than that of other U.S. pitviper species (Consroe et al. 1995), though this has been tempered by more recent work (Sánchez et al. 2003). The original package insert (Protherics Inc. 2008), which provided median effective dose (ED_{50}) comparisons among different rattlesnake species, suggested comparatively weak neutralization of *C. o. helleri* venom consistent with Consroe et al. (1995). However, the updated package insert (BTG International Inc. 2012) provided very different numbers suggesting comparable neutralizing effectiveness for *C. o. helleri* venom, but nevertheless stated that "higher doses may be required based on historical data." In spite of these *in vitro* assessments, *in vivo* analyses of clinical cases supported the conclusion that CroFab has similar effectiveness in treating *C. o. helleri* bites compared to other southern California species (Bush et al. 2002; Janes et al. 2010). Nevertheless, snake enthusiasts (at informal internet forums and meetings) and the media (e.g. Yong 2014) have perpetuated the belief that CroFab is ineffective in treating bites from this taxon.

The purpose of this study was to further elucidate and clarify the factors that affect both the etiology and clinical severity of venomous snakebites in southern California. The substantial data set also allowed us to compare the effectiveness of CroFab in treating envenomations from an unprecedented number of rattlesnake taxa (seven), including *C. o. helleri*. Because snake bite outcomes can be influenced by many variables, large sample sizes are required to ascertain which factors are most important while simultaneously controlling for potentially confounding variables. This study is based on one of the largest samples of snakebite cases collected from a single medical facility in the U.S. It also analyzes the largest number of dependent measures (four) and potentially explanatory factors (eight) using multivariate statistical models heretofore examined in a single study of snakebite severity.

Materials and Methods

Study Design

We retrospectively reviewed 354 medical records of snake bite victims that came to the emergency department of Loma Linda University Medical Center (LLUMC). Patient records were identified by a database search for records between 1990 and 2010 that contained the International Classification of Diseases, Ninth Revision (ICD-9), codes E905.0 (venomous snake and lizard bites) and 989.5 (toxic effect of venom). Some records lacking these codes were undoubtedly missed. We included patients if they were bitten by a venomous snake native to Southern California. We excluded patients that were bitten by animals other than venomous snakes, those bitten by venomous species not native to Southern California, and those whose envenomation did not result from a bite (one patient had an eye splashed by venom). For each patient, data were collected that covered the period of time between the bite and initial discharge. Information from follow-up visits was not included. The protocol was reviewed by the institutional review board and considered exempt from informed consent.

Data Collection

Abstractors included one of the investigators (AGC) and four research assistants, none of whom were blinded to goals of the study. The four research assistants were trained in data collection and calculation of snakebite severity by one of the investigators (AGC) via use of a standardized abstraction form. To assess consistency, all five abstractors reviewed 10 records and inter-rater reliability was determined using the one-way, agreement version of the intraclass correlation coefficient (ICC; Shrout & Fleiss 1979). By convention, ICC values of 0.0–0.20 indicate slight agreement, 0.21–0.40 indicate fair agreement, 0.41–0.60 indicate moderate agreement, 0.61–0.80 indicate substantial agreement, and 0.81–1.00 indicate almost perfect agreement (Landis & Koch 1977).

Calculating Snakebite Severity Scores

We calculated snakebite severity scores (SSS) using the rubric designed by Dart et al. (1996). This scoring method, which ranges from 0–20 points, is based on the objective evaluation of clinical parameters in six categories: local wound effects, hematologic (coagulation) parameters, and symptoms associated with the pulmonary, cardiovascular, gastrointestinal, and central nervous systems. The scores for each of these categories, which range from 0–3 or 0–4, were recorded separately and then summed to obtain a final score. Higher SSS scores indicate a more severe bite. If the patient record included no mention of an organ system abnormality relevant to any category, then that system was assumed to be unaffected by the snakebite. Because the SSS criteria published by Dart et al. (1996) were designed to assess adults, we adjusted the scoring of pulmonary and cardiac categories to account for differences in respiratory rate, heart rate, and blood pressure between children and adults (Table 2). These adjustments were based on other pediatric medical assessment rubrics and published data on normal values for pediatric vital signs (Tepas et al. 1987; Pollack et al. 1997; U.S. Department of Health & Human Services 2011; Fleming et al. 2011) in consultation with a pediatric emergency medicine specialist.

Table 2. Adjustments made to the pulmonary and cardiac subscores (SSsS) of the snakebite severity score (Dart et al. 1996) for pediatric patients; age ranges[a] are indicated across a row.

	Pulmonary Score		**Cardiac Score**				
	Respiration Rate (rpm)		**Heart Rate (bpm)**			**Systolic Hypertension (mmHg)**	**Systolic Hypotension (mmHg)**
Age Group:	**1-5**	**6-11**	**1-5**	**6-11**	**12-17**	**< 15**	**< 15**
SSsS 1	40	23	143	119	106	110+(age×2)	
SSsS 2	46	33	163	150	142		
SSsS 3	60	50	217	212	206		70+(age×2)[b]

Most values represent minimum cut-off values for a given level.
[a] Years of age
[b] For hypotension, scores less than or equal to this value were assigned to SSsS 3

We calculated two separate SSS scores for each case. Initial SSS (iSSS) was calculated by determining the maximum scores for each symptom category based on information recorded from time of the bite until the patient received their first dose of antivenom. Maximal SSS (mSSS) was determined by taking the maximum scores for each symptom category based on information recorded from the time of the bite until initial discharge from the hospital.

Determining Size of the Snake

We classified body size of the offending snake in 255 (72.0%) of the 354 cases. For many cases (15.3% of the 255), the usual treating physician (Sean P. Bush, SPB) recorded the length of the snake if it was brought to the hospital with the patient. In other cases (29.8%), the space between fang puncture wounds was recorded (fang spread). A formula (Equation 1; from Hayes et al., unpublished data) was applied to this number to arrive at an approximate length for the snake.

$$Snake\ Length\ (cm) = \frac{\log_{10}(Fang\ Spread(mm)) - 0.803}{0.006} \qquad (1)$$

Snake size was then grouped into the following categories based on length: small (< 40 cm), medium (40–75 cm), and large (>75 cm). For the remaining cases (54.9%), records of qualitative size assessment (e.g., "baby," "small," "large") from observers deemed reliable were used to assign these size categories.

Determining Species of Snake

We assigned species of snake for 204 (57.6%) cases. For 104 cases (51.0% of species identifications), SPB determined the species of snake that bit the patient from a specimen or photograph brought to the hospital with the patient. Otherwise, species assignment was based on detailed descriptions provided by reliable observers, as recorded in the medical record. The geographical range and preferred habitat of each snake species (Stebbins 2003; Campbell & Lamar 2004) were taken into account. If, for example, only one venomous species occurred at the geographic location of a bite, that species was assigned to the case.

Vials of Antivenom Administered

We determined the type and total number of vials of antivenom administered from the medical charts. Three kinds of antivenom were given to patients between 1990 and 2010: the equine-derived Antivenin (Crotalidae) Polyvalent (ACP; Wyeth-Ayerst Laboratories, Marietta, Pennsylvania, U.S.A; now part of Pfizer), CroFab (described previously), and Polyvalent Equine Anti-viper Serum (Antivipmyn®; Instituto Bioclon, Mexico City, Mexico).

We adjusted the total vials of antivenom to account for differences in the type of antivenom used. Most patients receiving Antivipmyn in this study did so as part of a multicenter clinical study (Bush et al. 2015) to test its safety and efficacy. Since the procedure for this clinical study set the dosage for Antivipmyn to be double that of CroFab, the number of Antivipmyn vials was divided by two to generate CroFab-equivalent vials. Although the median effective doses (ED_{50}) per rattlesnake species for Antivipmyn was roughly equivalent to CroFab (Sánchez et al. 2003), one vial of

Antivipmyn contained only about half the dry mass of active ingredient (500 mg) compared to CroFab (~1 g). We considered the number of vials of ACP and CroFab to be equivalent. Despite evidence that CroFab may be five-fold more effective than ACP based on ED_{50} values (Consroe et al. 1995), our analyses (see Results) failed to detect a significant difference between these two antivenoms in number of vials given.

Other Variables

We extracted several additional variables from each record, including patient mass, patient age at the time of the bite, sex of patient, date and time of the bite, time to hospital admission (elapsed hours between bite and admission to hospital), hospital duration (total hours between patient admission and initial discharge), limb bitten (upper or lower extremity), site of bite (distal or proximal to the wrist or ankle), and whether the patient had consumed alcohol or drugs just prior to the bite. We also recorded the type of interaction the patient had with the snake. If the patient saw the snake and his/her deliberate interaction with the snake caused the bite, then the interaction was deemed "illegitimate." If the interaction was not deliberate, and the patient did not see the snake prior to the bite, then the interaction was classed as "legitimate."

Statistical Analyses

We conducted most of the statistical tests described below using SPSS 13.0 (SPSS Inc., Chicago, IL), with standard defaults. For the one exception, we computed inter-rater reliability (ICC) using R 3.0.1 (R Core Team 2014) with the package irr (Gamer et al. 2012). We tested for parametric assumptions of normality, homoscedasticity, and linearity when appropriate, and applied $\log_{10}$ transformations as needed to better meet assumptions for all continuously-distributed variables except patient age. We set alpha at 0.05 for all tests. Following Nakagawa (2004), we chose not adjust alpha for multiple tests. Unless otherwise indicated, we report values as mean ± 1 S.E.

Effects of Season and Time of Day on Snakebite Occurrence

We utilized a one-sample chi-square (χ^2) test (Zar 1996) to examine seasonal differences in the number of envenomations per season. We categorized seasons as spring (March–May), summer (June–August), fall (September–November), and winter (December–February), and summed total bites per season across years. We also assessed differences in the proportion of snake size classes contributing to bites by season using a two-sample χ^2 test. The numbers of snakes in each size class were again summed across years for each season. We computed Cramer's *V* as a measure of effect size for these two tests, with values of 0.1, 0.3, and 0.5 loosely corresponding to small, medium, and large effects, respectively (Cohen 1988).

To determine whether time of day when bites occurred varied among seasons, we employed a Cochran-Mantel-Haenszel linear-by-linear association test (Mantel & Haenszel 1959). Seasons were categorized as mentioned above. The winter season was omitted from this analysis because few bites (N = 12) occurred during this season, and including it created four cells with expected values of less than five. Bite times were standardized to Pacific Standard Time, and then categorized as night (000–600 hours), morning (600–1200 hours), afternoon (1200–1800 hours), and evening (1800–2400 hours). We computed Pearson's *r* as a measure of effect size for this test, with values of 0.1, 0.3, and 0.5 loosely corresponding to small, medium, and large effects (Cohen 1988).

Antivenom Effectiveness

We employed an analysis of covariance (ANCOVA; Mertler & Vannatta 2004) model to compare the number of vials of ACP and CroFab administered to patients. This model used number of vials of antivenom (log_{10}-transformed) as the dependent variable, antivenom type as the independent variable, and mSSS (log_{10}-transformed) as the covariate. We used another ANCOVA model to test for differences in total vials of CroFab used among species. For this analysis, we only considered cases where CroFab was the sole antivenom administered to the patient and the species of envenomating snake was known. The model controlled for snake size as a fixed factor and patient mass (log_{10}-transformed) and mSSS as covariates. We computed ANCOVA effect sizes as partial η^2, with values of ~0.01, ~0.06, and > 0.14 loosely regarded as small, medium, and large effects, respectively (Cohen 1988). Although partial η^2 values tend to be upward biased (Pierce et al. 2004), the main effects and interactions never summed to more than 1.0 in our models, and therefore were not adjusted.

Factors Affecting Snakebite Severity

We relied on five multiple analysis of covariance (MANCOVA) models (Mertler & Vannatta 2004) to determine which factors affected the severity of a bite. We had to use multiple models because a single omnibus model that included all independent variables of interest resulted in too many empty cells. For each of these models, we used iSSS, mSSS, total vials of antivenom, and hospital duration (all log_{10}-transformed) as dependent variables. Cases in which patients received more than one type of antivenom were excluded from this analysis. From prior research (Wingert & Chan 1988; Blaylock 2004; Hayes et al. 2005; Benítez et al. 2007; Janes et al. 2010) and our own exploratory analyses, we controlled for three primary predictors in all five models: snake size (as a fixed factor), patient mass (covariate, not transformed), and patient age (covariate, not transformed). The remaining five secondary predictors entered into the models included: snake species, limb bitten (upper or lower), site of bite (proximity to wrist or ankle), interaction with snake (legitimate or illegitimate), and time to hospital admission (the first four as fixed factors, the latter as a covariate). To maximize sample size and statistical power, each of the secondary predictors was entered into a separate model with the three primary predictors, except that one model included only the primary predictors, and two variables (limb bitten and site of bite) were entered together in another model. We tested the assumption of homogeneity of regression slopes using separate MANCOVA models that included all interactions between the covariates and other predictors. No significant interactions were found, so we omitted interactions involving the covariates from our final models. We computed effect sizes as multivariate η^2, with values interpreted similar to those of η^2 (described previously).

Factors Affecting Site of Bite and Interaction with the Snake

We used binary logistic regression (Mertler & Vannatta 2004) to examine the influence of several factors on which limb was bitten (upper or lower), which site on the limb was bitten (proximal or distal to the ankle or wrist), and whether the bite was legitimate or illegitimate. To assess the factors associated with limb bitten and site of bite, we included snake size, patient sex, patient age, and whether or not the bite was illegitimate as predictors in a separate model for each dependent variable. To examine the factors associated with bite legitimacy, we included patient sex, patient age, alcohol or drug use, and size of snake as predictors. Odds ratios derived from the logistic regression models were calculated as measures of effect size.

Results

Demographics and Bite Characteristics

Of the 354 cases that were reviewed, 285 (80.5%) involved male patients and 69 (19.5%) were females. The median age was 34.4 (range: 1 to 81) years. Ninety-seven patients (27.4%) were under age 18, while 33 patients (9.3%) were over age 60. We were able to determine the anatomical location of the bite for all cases, and found that 245 (69.2%) were to an upper limb, and 311 (88.0%) were distal to the wrist or ankle. No bites were delivered to the head or trunk. Of the 308 cases for which a determination could be made, 45.8% were classed as illegitimate. Sixty-four (18.0%) of the patients admitted to using alcohol or drugs just prior to the bite. Median time between the time of the bite and hospital admission was 1 hr (range: 4.8 min to 42 hr). The average hospital duration was 50.9 hr (95% CI: 46.4 to 55.5 hr). The mean iSSS and mSSS were 4.6 (95% CI: 4.3-4.9; range: 0–18) and 7.0 (95% CI: 6.6–7.3), respectively. There were three recorded deaths (0.8% of all cases). Seven rattlesnake taxa inflicted bites (Table 1), with the majority (26.8%) caused by Southern Pacific Rattlesnakes.

Effects of Season and Time of Day on Snakebite Occurrence

The frequency of bites differed among seasons ($\chi^2 = 103.52$, df = 3, $P < 0.001$), with the majority (335, 95%) occurring from March to October, during the active season of southern California's rattlesnakes (Fig. 1). The proportion of snakes of different size classes delivering bites also varied among seasons (χ^2 = 37.66, df = 6, P < 0.001, Cramer's V = 0.27). There was a large pulse of bites during spring, of which 55.6% resulted from small snakes and 44.4% from medium and large snakes. Another pulse occurred in fall, which included a substantial proportion of bites from newly born and other small snakes (81.6%), and a much smaller proportion of medium and large snakes (18.3%).

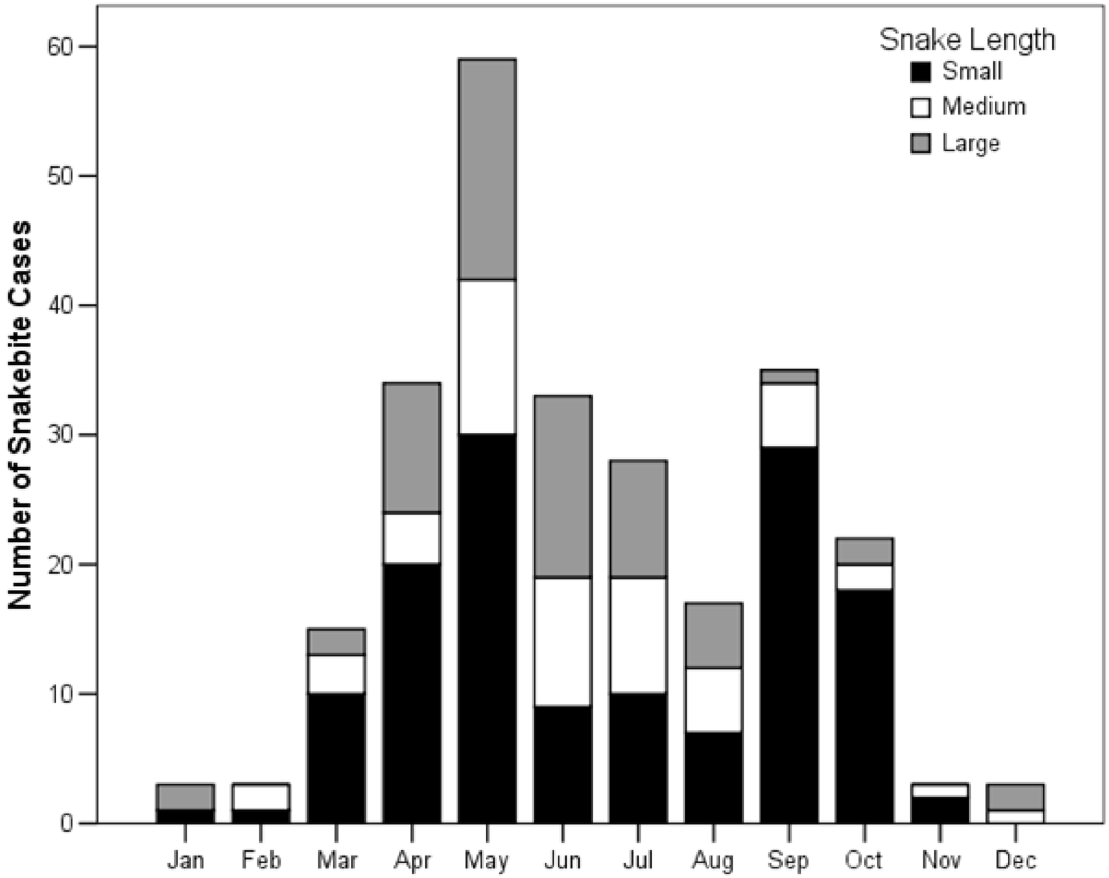

Figure 1. Rattlesnake envenomation cases by month and snake size. Total number of rattlesnake snakebite cases per month presenting at the Loma Linda University Medical Center between 1990 and 2010, and proportions of bites from three snake length classes (small: < 40 cm; medium: 40–75 cm; large: >75 cm).

The frequency of bites differed by time of day ($\chi^2 = 130.52$, df = 3, $P < 0.001$). Most bites happened during the afternoon (168, 47.7%), fewer bites occurred during the morning (94, 26.7%), still fewer bites occurred in the evening (71, 20.2%), and the fewest bites happened at night (19, 5.4%). There was a significant association between the time of day a bite occurred and season (omitting winter [see above]; Mantel-Haenszel $\chi^2 = 7.91$, df = 1, $P = 0.005$, n = 340). Pearson's r (0.15) suggested a direct relationship, with more bites happening later in the day as the year progressed from spring to fall.

Antivenom Effectiveness

Table 3 provides information on the amount of antivenom used. The majority of patients (244, 68.9%) received CroFab. Seventy-one patients (20.1%) received ACP, which was the only FDA-approved antivenom for North American pitvipers prior to October 2000, when CroFab received FDA approval. Ten patients (2.8%) received Antivipmyn as part of the aforementioned clinical study. Eleven patients (3.1%) received both ACP and CroFab during the period between 2001 and 2003, when CroFab was replacing ACP in the market. One patient received both CroFab and Antivipmyn on an experimental basis to treat a severe envenomation. Twelve patients (3.4%) received no antivenom due to minimal or no envenomation. The remaining five patients (1.4%) were enrolled in the Antivipmyn clinical study, but information was not released regarding the type of antivenom they received. We found no significant difference in number of vials of ACP and CroFab administered to patients ($F_{2,333} = 0.21$, $P = 0.82$, partial $\eta^2 = 0.001$, $n = 337$; note the miniscule effect size).

Table 3. Vials of antivenom administered, by type, to snakebite patients.

Antivenom Type	N	Mean Number of Vials (±SE)	Range
Antivenin Crotalidae Polyvalent (ACP)	71	18.55 (±2.04)	1–100
CroFab	244	14.4 (±0.54)	2–66
Antivipmyn	10	22.4 (±3.51)	10–46
Multiple Types[a]	12	—	—
None	12	—	—
Unknown[b]	5	—	—

[a] Values not informative
[b] Enrolled in blind study

Table 4 provides the unadjusted and estimated marginal mean (adjusted for snake size, patient mass, relative bite severity) vials of CroFab for each species. The species with lowest unadjusted means was *C. cerastes*, which averages substantially smaller in body size than all other taxa (Klauber 1956; Campbell & Lamar 2004). When controlling for snake size, patient mass, and mSSS in the ANCOVA model, mSSS exerted the largest effect on number of antivenom vials ($F_{1,108} = 34.34$, $p < 0.001$, partial $\eta^2 = 0.241$), as expected. The main effect of species approached significance with a moderate effect size ($F_{6,108} = 2.06$, $p = 0.064$, partial $\eta^2 = 0.10$), whereas snake size was significant in spite of the smaller effect size ($F_{2,108} = 3.19$, $p = 0.045$, partial $\eta^2 = 0.06$) due to fewer degrees-of-freedom. In general, bites from larger snakes required more vials of CroFab, but the interaction between species and snake size ($n = 130$, $F_{11,108} = 2.22$, $p = 0.018$, partial $\eta^2 = 0.18$) indicated that the relationship between snake size and number of antivenom vials was stronger for some snake taxa than others (data not shown). The species with the lowest marginal means (*C. atrox* and *C. scutulatus*) were two of the four species whose venom is used to produce CroFab. Bites inflicted by *C. o. helleri* required antivenom quantities similar to those of other species. Patient mass was not significant in this model ($F_{1,108} = 2.49$, $p = 0.117$, $\eta^2 = 0.02$).

Table 4. Mean and estimated marginal mean number of vials of CroFab (antivenom) administered for each species.

	Mean Vials of Antivenom			Estimated Marginal Means		
Species	N	Mean	95% CI	N	Mean	95% CI
Mohave Rattlesnake	21	13.10	9.36–16.83	18	10.49	8.31–13.17
Northern Pacific Rattlesnake	4	12.50	-1.58–26.58	4	14.79	9.27–23.28
Red Diamond Rattlesnake	16	17.13	12.07–22.18	13	14.27	10.1–19.99
Sidewinder Rattlesnake	19	9.32	7.21–11.42	15	12.69	9.03–17.69
Southern Pacific Rattlesnake	75	15.52	13.14–17.90	64	12.71	11.34–14.23
Speckled Rattlesnake	10	19.50	14.46–24.54	9	17.44	12.91–23.46
Western Diamondback Rattlesnake	8	11.25	5.60–16.90	7	8.81	5.85–13.04

Estimated marginal means are based on an ANCOVA model (with type IV sum of squares due to no data in one cell) that controlled for snake size as a fixed factor, and log_{10}-transformed patient mass and maximal snakebite severity score (mSSS) as covariates. Marginal means computed at the following log_{10}-transformed values: patient mass = 1.83, maximal SSS = 0.89. Main effects were significant for snake size and mSSS, and approached significance for snake species, but a significant interaction existed between species and snake size (see text for further details).

Inter-rater Reliability for Calculating SSS

Inter-rater reliability (ICC) values for total antivenom (ICC = 0.93, 95% CI: 0.84–0.98) and hospital duration (ICC = 0.97, 95% CI: 0.92–0.99) showed almost perfect agreement between abstractors. Corresponding values for iSSS (ICC = 0.64, 95% CI: 0.37–0.88) and mSSS (ICC = 0.65, 95% CI: 0.40–0.89) were lower, but still showed substantial agreement.

Factors Affecting SSS

Of the eight independent variables and cofactors used among the five MANCOVA models (snake size, snake species, patient age, patient mass, limb bitten, site of bite, interaction with snake, time to hospital admission), only snake size and patient mass showed significance (Table 5). Snake size was significant in Models 1, 3, 4, and 5

(Model 1: Wilks' $\Lambda = 0.78$, $F_{8,480} = 7.87$, $P < 0.001$, multivariate $\eta^2 = 0.12$; Model 3: Wilks' $\Lambda = 0.933$, $F_{8,462} = 2.03$, $P = 0.034$, multivariate $\eta^2 = 0.03$; Model 4: Wilks' $\Lambda = 0.859$, $F_{8,474} = 4.69$, $P < 0.001$, multivariate $\eta^2 = 0.07$; Model 5: Wilks' $\Lambda = 0.785$, $F_{8,478} = 7.67$, $P < 0.001$, multivariate $\eta^2 = 0.11$), but only approached significance in Model 2 when species of snake was included as a factor (Wilks' $\Lambda = 0.90$, $F_{8,276} = 1.85$, $P = 0.067$, multivariate $\eta^2 = 0.05$). Patient mass was significant in all five models (Model 1: Wilks' $\Lambda = 0.91$, $F_{4,240} = 5.77$, $P < 0.001$, multivariate $\eta^2 = 0.09$; Model 2: $\Lambda = 0.90$, $F_{4,138} = 3.97$, $P = 0.004$, multivariate $\eta^2 = 0.10$; Model 3: $\Lambda = 0.92$, $F_{4,231} = 4.93$, $P < 0.001$, multivariate $\eta^2 = 0.08$; Model 4: $\Lambda = 0.91$, $F_{4,209} = 5.29$, $P < 0.001$, multivariate $\eta^2 = 0.09$; Model 5: $\Lambda = 0.91$, $F_{4,239} = 5.69$, $P < 0.001$, multivariate $\eta^2 = 0.09$).

Follow-up univariate ANCOVA results for model 1 confirmed the importance of snake size and patient mass. Snake size significantly affected all four dependent variables (iSSS: $F_{2,243} = 7.20$, $P = 0.001$, partial $\eta^2 = 0.06$; mSSS: $F_{2,243} = 17.40$, $P < 0.001$, partial $\eta^2 = 0.13$; total antivenom: $F_{2,243} = 18.54$, $P < 0.001$, partial $\eta^2 = 0.13$; hospital duration: $F_{2,243} = 14.69$, $P < 0.001$, partial $\eta^2 = 0.11$). Patient mass significantly influenced mSSS ($F_{1,243} = 4.77$, $P = 0.030$, partial $\eta^2 = 0.02$), total antivenom ($F_{1,243} = 3.92$, $P = 0.049$, partial $\eta^2 = 0.02$), and time to hospital admission ($F_{1,243} = 7.75$, $P = 0.006$, partial $\eta^2 = 0.03$), but not iSSS ($F_{1,243} = 0.99$, $P = 0.32$, partial $\eta^2 = 0.004$). In each model, snake size explained substantially more variance (effect sizes 0.06–0.13) than patient mass (effect sizes ≤0.03). Figure 2 illustrates the positive relationship between snake size and snakebite severity, and the inverse relationship between patient mass and snakebite severity (note that no significant interactions existed between snake size and patient mass).

Table 5. Results (*P*-values and multivariate η^2 effect sizes) of multivariate analysis of covariance (MANCOVA) models for measures of clinical severity resulting from rattlesnake bites in southern California. Values in bold font are significant.

Independent variables	**Model 1 (*N* = 248)**		**Model 2 (*N* = 163)**[a]		**Model 3 (*N* = 248)**		**Model 4 (*N* = 220)**		**Model 5 (*N* = 248)**	
	P	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**
Snake size	**<0.001**	0.12	0.067	0.05	**0.042**	0.03	**<0.001**	0.07	**<0.001**	0.11
Patient mass	**<0.001**	0.09	**0.004**	0.10	**0.001**	0.08	**<0.001**	0.09	**<0.001**	0.09
Patient age	0.281	0.02	0.192	0.04	0.137	0.03	0.480	0.01	0.314	0.02
Snake species	–	–	0.750	0.03	–	–	–	–	–	–
Snake species × snake size	–	–	0.226	0.08	–	–	–	–	–	–
Upper vs. lower limb	–	–	–	–	0.533	0.01	–	–	–	–
Proximal vs. distal bite	–	–	–	–	0.515	0.01	–	–	–	–
Proximal or distal bite × snake size	–	–	–	–	0.709	0.01	–	–	–	–
Upper or lower limb × snake size	–	–	–	–	0.085	0.03	–	–	–	–
Proximal or distal × upper or lower limb	–	–	–	–	0.323	0.02	–	–	–	–
Proximal or distal × upper or lower limb × snake size	–	–	–	–	0.298	0.02	–	–	–	–
Interact with snake	–	–	–	–	–	–	0.477	0.01	–	–
Interact × snake size	–	–	–	–	–	–	0.275	0.02	–	–
Time to hospital admission	–	–	–	–	–	–	–	–	0.617	0.01

Dependent variables: initial snakebite severity score (iSSS), maximal snakebite severity score (mSSS), vials of antivenom, hospital duration (all were $\log_{10}$-transformed).

Independent variables: all treated as fixed factors except that patient mass and patient age (both covariates)

[a] Type IV sum of squares model due to one empty cell (similar to results from type III sum of squares model).

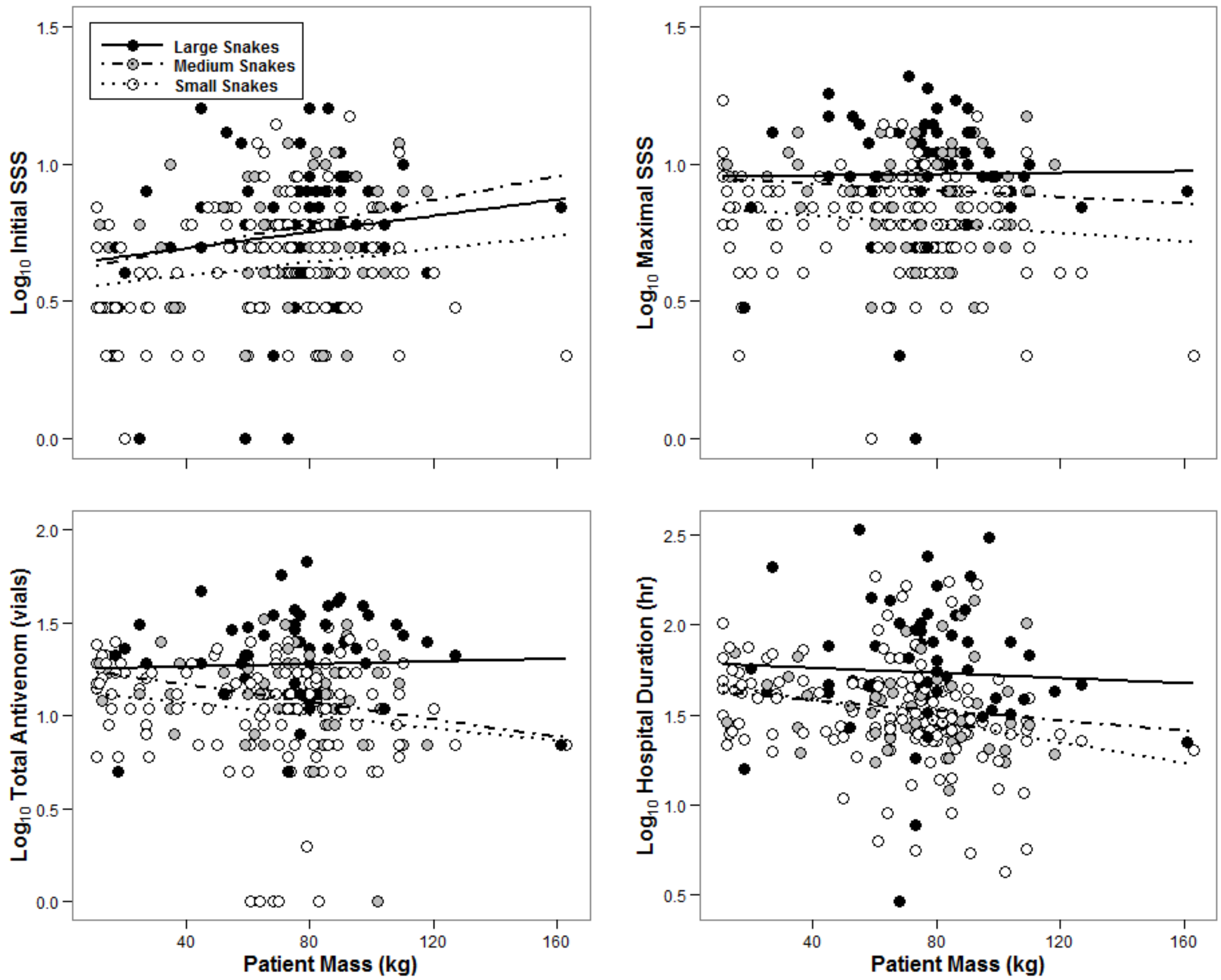

Figure 2. Relationship of snakebite severity to snake size and patient mass. Relationships between four measures of rattlesnake snakebite severity (snakebite severity score [SSS] at initial presentation and at maximal severity, number of vials of antivenom, hospital duration), snake size, and patient mass. Note the $\log_{10}$-transformed measures. Analysis of covariance (ANCOVA) models indicated that snake size was positively associated with all four measures of snakebite severity (all $P \leq 0.001$, partial $\eta^2 = 0.06$–0.13). Patient mass was independent of initial SSS ($P = 0.32$, partial $\eta^2 = 0.004$), but negatively associated with the other three measures of snakebite severity ($P = 0.006$–0.049, partial $\eta^2 = 0.02$–0.03). $N = 249$–252 for each model.

Factors Affecting Location of Bite and Interaction with Snake

Bites to Upper vs. Lower Extremities

The logistic regression model significantly distinguished between bites to upper and lower limbs (69.2% and 30.8% of all cases, respectively; $P < 0.001$, Table 6). This model successfully predicted which type of limb was bitten with moderate success (78.9% of cases). Two predictors were significant: sex of the patient ($P = 0.012$) and interacting with the snake (i.e., legitimacy of bite; $P < 0.001$). Odds ratios derived from the logistic regression model indicated that males were 2.9 times more likely to sustain bites to the upper limb than females (75.1% and 44.9% of cases, respectively), and that illegitimate bites were 111 times more likely than legitimate bites to be to the upper rather than the lower limb (97.9% and 46.1% of cases, respectively). The limb that was bitten was independent of size of snake and age of patient.

Proximal vs. Distal Bites

This logistic regression model significantly distinguished between bites to the proximal and distal portions of the limbs (12.1% and 87.9% of all cases, respectively; $P < 0.001$, Table 6). The model predicted site of bite with excellent success (87.2% of cases). Again, two predictors were significant: size of the snake ($P = 0.002$) and interacting with the snake (i.e., legitimacy of bite; $P = 0.001$). A cross-tabulation revealed that 58.4%, 19.9%, and 21.7% of distal bites were from small, medium, and large snakes, respectively, whereas 17.2%, 31.0%, and 51.7% of proximal bites were from small, medium, and large snakes, respectively. A Mantel-Haenszel χ^2 test for trend proved significant ($\chi^2_1 = 18.380$, $P < 0.001$), suggesting that smaller snakes were more likely to deliver distal bites, whereas larger snakes were more likely to deliver proximal bites.

Table 6. Results of logistic regression models examining factors predicting bites to upper versus lower limb, bites distal or proximal to the wrist or ankle, and whether the bite was incidental (legitimate) or involved deliberate interaction with the snake (illegitimate).[a]

Predictors	**B**	**SE**	**Wald**	***P***	**Exp(B)**
Upper vs. Lower Limb					
Snake size	-	-	4.557	0.102	-
Patient sex[b]	1.070	0.426	6.308	**0.012**	2.915
Patient age	-0.004	0.009	0.174	0.676	0.996
Illegitimate vs. legitimate bite[c]	4.710	1.027	21.049	**<0.001**	111.004
Distal vs. Proximal					
Snake size	-	-	12.196	**0.002**	-
Patient sex[b]	-0.817	0.600	1.852	0.174	0.442
Patient age	-0.004	0.011	0.153	0.695	0.996
Illegitimate vs. legitimate bite[c]	1.963	0.575	11.638	**0.001**	7.143
Illegitimate vs. Legitimate					
Snake size	-	-	1.052	0.591	-
Patient sex[b]	1.124	0.389	8.348	**0.004**	3.076
Patient age	0.012	0.008	2.420	0.120	1.012
Alcohol or drug use[c]	1.733	0.460	14.224	**<0.001**	5.660

[a] Upper vs. lower limb (coded as one and zero, respectively): χ^2_5 = 105.320, P < 0.001, -2 log likelihood = 173.521, Nagelkerke R^2 = 0.525, 78.9% predicted correctly. Proximal vs. distal (coded as one and zero, respectively): χ^2_5 = 33.476, P < 0.001, -2 log likelihood = 139.995, Nagelkerke R^2 = 0.257, 87.2% predicted correctly. Illegitimate vs. legitimate (coded as one and zero, respectively): χ^2_5 = 32.476, P < 0.001, -2 log likelihood = 279.879, Nagelkerke R^2 = 0.178, 66.1% predicted correctly.

[b] Males coded as one, females as zero

[c] Illegitimate bites coded as one, legitimate bites as zero

[d] Alcohol or drugs use coded as one, no alcohol or drug use coded as zero

Odds ratios derived from the logistic regression model indicated that illegitimate bites were 7.1 times more likely than legitimate bites to occur distal to the ankle or wrist rather than more proximally (95.7% and 80.8% of cases, respectively). Site of the bite was independent of sex and age of the patient.

Legitimate vs. Illegitimate Bites

This final logistic regression model provided significant discrimination between legitimate and illegitimate bites (54.2% and 45.8% of 308 cases, respectively; $P < 0.001$, Table 6). However, its prediction success was more moderate (66.1%). This model revealed that sex of the patient ($P = 0.004$) and alcohol or drug use ($P < 0.001$) were significant predictors. Odds ratios derived from the logistic regression model showed that males had a 3.1-fold greater chance of sustaining an illegitimate bite than females (52.8% and 21.3% of cases, respectively), and those patients who admitted using alcohol or drugs prior to the bite were 5.7 times more likely to experience an illegitimate bite than a legitimate bite (84.9% and 37.6% of cases, respectively). Interacting with the snake was independent of snake size and patient age.

Discussion

We conducted this study, in large part, to identify and better manage the risks associated with venomous snakebite in a heavily populated region of the U.S. Norris and Bush (2007) remarked that a common clinical profile for a snakebite victim in the U.S. is a “young, intoxicated male bitten on the hand while intentionally interacting with the snake.” Our study largely supports this contention by showing that being male and being intoxicated significantly increase one’s risk of sustaining an illegitimate bite, and that illegitimate bites are more likely to be to the upper limb and distal to the ankle or wrist. Our findings also indicate that the major factors that affect the clinical severity of a venomous snakebite are size of the snake, with larger snakes causing more severe bites, and mass of the victim, with greater mass mitigating bite severity. Here, we discuss both the etiology of venomous snakebite, particularly in relation to legitimate and illegitimate bites, and the diverse factors that influence the clinical severity of venomous snakebite.

Etiology

The etiological profile of snakebite victims in the U.S. seems to have changed since the beginning of the twentieth century. The earliest studies, which sought to characterize venomous snakebites for the entire U.S., suggested that most bites were accidental and with fewer than half of bites being to the upper limb. Willson (1908) reported 42.7% of U.S. bites being to the upper limb. Likewise, Hutchison (1930) reported 42.9% of bites being to the upper limb and only 6.3% of bites resulting from catching venomous snakes or handling captive ones. Later on, Parrish (1966), in a large study characterizing 3,367 bites which occurred between 1958–1959 from the entire U.S., reported 38% of bites being to the upper limbs. However, other studies conducted by Parrish covering the same time period indicate some regions where the majority of bites were to the upper limbs. In New England, 8 of 12 cases (66.7%) involved bites to the upper limb (Parrish et al. 1960), and 95 of 146 snakebites in California (65%) were to the upper extremities, with 23 (15.7%) of all bites occurring when people were handling a venomous snake (Parrish et al. 1964). Later, Russell (1980) estimated that 25% of bites in the U.S. were illegitimate.

With few exceptions (Dart et al. 1992; Forrester & Stanley 2004; Correa et al. 2014; Gerardo et al. 2015), more recent studies report a greater percentage of illegitimate bites and a majority of bites to the upper limb. Most are from individual hospitals in regions of the U.S. where snakebites are relatively common, particularly the east (Rudolph et al. 1995; Morandi & Williams 1997; Thorson et al. 2003) and southwest (Wingert & Chan 1988; Curry et al. 1989; Downey et al. 1991; White & Weber 1991; Plowman et al. 1995; Tokish et al. 2001; Tanen et al. 2001; Janes et al. 2010; Spano et al. 2013), though one covers cases across the entire U.S. (O'Neil et al. 2007). In a study of 30 cases from West Virginia, Morandi & Williams (1997) found that 70% of bites were to upper limbs and 67% of bites were illegitimate (40% of these were from rattlesnake round-ups). This study also found that 95% of illegitimate bites were to the upper extremities. Similarly, Curry et al. (1989), in a study of 85 cases from Arizona, found that 74.4% were to upper limbs and 56.7% of cases were illegitimate. This study further found that only 73% of bites to upper extremities were illegitimate, whereas all bites to the lower extremity were legitimate. Studies from Southern California are similar. Wingert & Chan (1988) found that, of 282 cases, 87% were to upper limbs and 57% were illegitimate, and Janes et al. (2010), using 142 cases (also from LLUMC), found that 70% of bites were to upper limbs and 67% of bites were illegitimate. The current study is consistent with the recent trend of bites resulting from interactions with snakes. We found that 69.2% of cases involved bites to the upper limb, and though we found a smaller proportion of illegitimate bites (45.7% of classifiable cases), it is much higher than that reported for the earliest studies. Consistent with other studies (Curry et al. 1989; Morandi & Williams 1997; O'Neil et al. 2007), we also found a significant relationship between bites to the upper limbs and illegitimate bites, with illegitimate bites being 111.0 times more likely to be to upper limbs.

One bite characteristic that has changed little since the early 1900s is whether a bite was inflicted proximal or distal to the ankle or wrist. All previous literature has reported a majority of distal bites. Among the earlier reports, Willson (1908) reported 76.3% distal bites, and Hutchison (1929) reported 77.8% distal bites. More recently, LoVecchio & DeBus (2001) reported that 67% of the children in his study sustained distal bites, and Thorson et al. (2003) reported that 88% of Copperhead (*Agkistrodon contortrix*) bites in the Carolinas were to distal portions of the extremities. The latter study uncovered a relationship between more distal bites and those received illegitimately that we confirmed in ours.

Another trend that has not changed is the proportion of males that are envenomated. The earliest study reporting this (Willson 1908) found that 74.5% of 740 cases were to males. With the exception of one study in which 47% of victims were male (Gerardo et al. 2015), more recent studies continue to show a majority of male victims, with proportions ranging between 54–93% (Christopher & Rodning 1986; Wingert & Chan 1988; Curry et al. 1989; Downey et al. 1991; White & Weber 1991; Plowman et al. 1995; Rudolph et al. 1995; Morandi & Williams 1997; LoVecchio & DeBus 2001; Tanen et al. 2001; Tokish et al. 2001; Thorson et al. 2003; Forrester & Stanley 2004; Corneille et al. 2006; O'Neil et al. 2007; Seifert et al. 2009; Janes et al. 2010; Lavonas et al. 2011; Spano et al. 2013). Our value of 80.5% is within this range. Our finding that males are more likely than females to sustain illegitimate bites is consistent with Curry et al. (Curry et al. 1989), who found that 97.9% of illegitimate bites were sustained by males compared to 72.2% of legitimate bites.

The proportion of snakebite cases associated with intoxication is inconsistent in the literature. Our study found that intoxication (alcohol or drugs) was involved in 18.0% of cases. This proportion is similar to that of several prior U.S. studies (13.9%, Downey et al. 1991; 17.2%, Janes et al. 2010). However, others have reported higher levels of alcohol use: Wingert & Chan (1988) reported 28% of cases from Southern California, Curry et al. (1989) 38.6% from Arizona, and Morandi & Williams (1997) 40% from West Virginia. In stark contrast, one central California study reported 7% (of 46 cases; Spano et al. 2013). Some variation may result from different methods used to ascertain intoxication. Our finding that intoxicated individuals are 5.6 times more likely to sustain an illegitimate bite is consistent with Curry et al. (1989), who found that 56.5% of illegitimate bite cases were under the influence of alcohol compared to 16.7% of legitimate bites.

The low frequency of dry bites and/or minimal envenomation (3.6%), which can be difficult to differentiate, contrasts with the higher percentages reported elsewhere for rattlesnakes (see Hayes et al. 2002 and Hayes 2008 for reviews). However, many patients represented in our dataset were transferred to LLUMC from other medical facilities. Since, these facilities were unlikely to transfer patients that did not show symptoms of envenomation, our percentage should not be considered representative of the actual rate of dry bites in southern California.

Our study further suggests that most rattlesnake bites in southern California occur during the afternoon hours of the spring and fall. Our finding that most bites occur in the afternoon is consistent with previous studies (Parrish et al. 1964; Parrish 1966; Curry et al. 1989; Plowman et al. 1995). Our finding that bites tend to occur later in the day as the season progresses has not been reported before, and may be related to the increase in day-length and ambient temperature as one moves from spring into summer. However, why the trend should continue into fall, when day-length is decreasing, remains unclear. Our finding that envenomations peak in spring and fall is at odds with most of the literature. Several studies from various regions of the U.S. (Ennik 1980; Downey et al. 1991; Plowman et al. 1995; Rudolph et al. 1995; Spano et al. 2013) and two large epidemiological studies covering the entire U.S. (Parrish 1966; Seifert et al. 2009) reported venomous snakebite incidence peaking in the summer (June and July). However, two studies from Arizona found that bite incidence peaked in the fall (September and October; LoVecchio & DeBus 2001; Hardy 1986), and another epidemiological study from Texas reported that bites peak in the spring (May; Forrester & Stanley 2004). The study most relevant to ours is that of Parrish et al. (1964), which documented two snakebite peaks in California, with one in the spring (May) and the second at the end of the summer (August). These seasonal differences in the incidence of venomous snakebite are likely related to substantial seasonal variation among rattlesnake species in movement patterns associated with the timing of the reproductive cycle (Aldridge & Duvall 2002; Schuett et al. 2002), and seasonal shifts in snake activity from diurnal to nocturnal in the hotter regions of the U.S. Most medically significant venomous snakes in the U.S. are pit vipers, which exhibit prolonged mate searching polygyny in which males significantly increase their movements in search of females (Duvall et al. 1992; Aldridge & Duvall 2002), and in the process may be more likely to encounter humans. Indeed, some evidence suggests that male rattlesnakes cause the majority of human envenomations (Cardwell et al. n.d.). Some species mate during just one season, either in spring or late summer/early fall, whereas other species mate in both seasons (Aldridge & Duvall 2002; Schuett et al. 2002). The somewhat unique seasonal distribution of snake bites in our study likely results from most rattlesnake species in southern California mating during both spring and late summer/fall (Aldridge & Duvall 2002; Cardwell 2008; Dugan et al. 2008; Brown et al. 2009; Dugan 2011; Chapter 2 this dissertation), including the species causing the most bites in our study, the Southern Pacific Rattlesnake (*C. o. helleri*; Dugan et al. 2008), and the extreme summer temperatures that shift snake activity to nocturnal hours when humans are less likely to encounter them. Parturition in U.S. pitviper species, including southern California species, also happens during the fall (Aldridge & Duvall 2002), and the dispersal of young snakes after this event likely explains the high proportion of bites (81.6%) attributed to small snakes during this period.

Effectiveness of CroFab in Treating Southern California Rattlesnake Bites

Our analysis did not detect statistically significant species differences in the number of vials of CroFab needed to resolve rattlesnake envenomation. However, the moderate effect size that approached significance for estimated marginal means of species (Table 4) suggests that CroFab may be most effective against the venoms of the two southern California species that are used in the manufacture of CroFab (*C. atrox* and *C. scutulatus*).

Our results do not support the ongoing contention of amateur herpetologists (on internet discussion groups) and the media (Yong 2014) that CroFab is comparatively ineffective at treating bites from *C. o. helleri*. This contention was fueled in part by a prior study of CroFab's constituents showing exceptionally high ED_{50} values for *C. o. helleri* relative to nine other North American crotaline taxa (Consroe et al. 1995). However, a later study (Sánchez et al. 2003) determined that CroFab had moderate effectiveness against *C. o. helleri* compared to 16 other North American taxa. The original product insert by Protherics provided an ED_{50} of 122 mg antivenom/mg venom for *C. o. helleri*, compared to 8 and 15, respectively for *C. adamanteus* and *C. scutulatus* (two species used in the production of CroFab; Protherics Inc. 2008). More recently, the package insert was changed, inexplicably, to provide ED_{50} values of 6 for *C. o. helleri* and 18 for both *C. adamanteus* and *C. scutulatus* (BTG International Inc. 2012). Regardless, our results suggest that CroFab is equally effective at treating *C. o. helleri* envenomations compared to other southern California rattlesnakes. Earlier, Bush et al. (2002) demonstrated efficacy of CroFab against *C. o. helleri* venom in a clinical setting, but with a much smaller sample.

Factors Influencing Clinical Severity

This study addresses many of the factors thought to influence the severity of bites from venomous snakes. These factors have been divided into two categories: factors related to the snake and factors related to the human victim (Hayes & Mackessy 2010). The two major factors associated with the snake include the amount of venom injected and the toxicity of the venom. The amount of venom injected is, in turn, thought to be influenced by several things, including amount of venom present in the snake's glands and the level of threat it perceives. Despite a persistent myth in the U.S. that baby rattlesnakes inject more venom and inflict more severe bites (Hayes et al. 2002), evidence strongly supports the notion that the major factor determining the amount of venom present in the glands of a snake is its size. As a rattlesnake grows, the amount of venom in its gland increases exponentially (Glenn & Straight 1982; Hayes 1991; Mackessy et al. 2003). Because larger snakes have more venom, it would be expected that they inject more venom and subsequently cause more severe bites. Our findings support this hypothesis and are consistent with other studies (Wingert & Chan 1988; Thomas et al. 1998; Hayes et al. 2005; Janes et al. 2010). In fact, snake size was the single most important predictor of snakebite severity, explaining roughly 6–13% of the variation in SSS.

Evidence further suggests that venomous snakes are able to meter their venom, regulating the amount they inject depending on context (Hayes 2008). One context that may trigger a snake to inject more venom is when it perceives a significant threat. Indeed, studies show that some snake species will inject more venom when grasped by a human than when unrestrained (Hayes et al. 2002). Although one might expect illegitimate bites to be more severe than legitimate ones due to potentially greater provocation of the snake, all bites result from contact with the snake or the threat of close proximity, so it's not surprising—from the snake’s perspective—that the current study found no differences in severity of legitimate and illegitimate bites (see also Janes et al. 2010). The limited data for rattlesnakes suggests that they do not inject more venom when grasped (Herbert 1998; Rehling 2002).

Considerable research has examined differences in venom composition among venomous snakes in the U.S. Venom variation has been documented taxonomically, geographically, and ontogenetically (Chippaux et al. 1991). However, much less work has been done looking at the clinical significance of these venom variations (Janes et al.

2010; Massey et al. 2012). Despite the wide range of toxicities in the venoms of the different species of rattlesnakes native to southern California (intravenous mouse LD_{50}: *C. scutulatus* ~0.14 mg/kg, *C. ruber* ~3.51 mg/kg; Glenn & Straight 1982), we detected no differences in snakebite severity (SSS) among species in this study (see also Hayes et al. 2005; Janes et al. 2010). The reason for this remains unclear, but it does call into question the relevance of overall toxicity (LD_{50} values in mice) in the clinical severity of rattlesnake bites. Currently, we are investigating whether the six clinical symptoms (snakebite severity subscores) differ among taxa to determine whether interspecific venom differences produce different clinical syndromes, which might necessitate different treatment algorithms (Appendix 1).

In terms of factors related to the human victim, we found a small but significant effect of patient mass (explaining <3% of variation in SSS), with larger patents experiencing less severe bites. This outcome differs from some (Wingert & Chan 1988; Janes et al. 2010) but not all (Hayes et al. 2005) southern California data sets. The conclusions of studies outside of southern California have also been mixed. Pinho et al. (2005) found that those envenomated by the South American rattlesnake (*C. durissus*) were more likely to experience acute renal failure if they had a small body surface, whereas Parrish et al. (Parrish et al. 1965) concluded that there were no real differences in bite severity among patient age groups (a factor closely related to patient mass) in his study of snakebite victims from 10 U.S. states. Small effect size may help explain why some previous studies have failed to detect this relationship, yet these studies also have other limitations. Wingert & Chan (1988) did not utilize statistical models that accounted for potentially confounding variables such as snake size, whereas Janes et al. (2010) did not adjust their use of the Dart et al. (1996) snakebite severity score when considering pediatric patients. One significant limitation of Parrish et al. (1965) is that their conclusion was not based on any statistical test. In fact, a re-analysis of the data they presented leads to a different conclusion. We applied a Mantel-Haenszel linear-by-linear χ^2 test to the data in table V of Parrish et al. (1965) showing the number of snakebite cases cross-tabulated by bite severity grade and age group. Omitting cases wherein the severity grade was omitted, our analysis yielded a significant result (Mantel-Haenszel $\chi^2 = 4.66$, df = 1, $P = 0.031$). The associated negative Pearson's r (-0.06) suggested an inverse relationship between age group and bite severity, which is consistent with the results of our study. Because our results match what might be expected theoretically (Russell 1983), and are based on methodologies which overcome the limitations of previous studies that have failed to detect this relationship, we are confident in our conclusions. Moreover, the negative relationship between patient size and snakebite severity has implications for the dosing of antivenom in pediatric patients (Chapter 4 this dissertation).

Another potential contributing factor to snakebite severity is the anatomical location of the bite. Moss et al. (1997) presented evidence that bites distal to the first interphalangeal joint of the fingers or toes tend to be less severe than bites proximal to these joints, and attributed the difference to the distal digit's smaller volume and reduced blood supply. However, the authors did not consider snake size in their analysis. Gerardo et al. (2015) reported a difference in the amount of antivenom needed to treat bites to upper vs. lower extremities. However the authors did not report which extremities need more antivenom and, as with the previous study, did not consider snake size in their analysis. Our results revealed that bites to the distal limbs are more likely to be caused by small snakes than large snakes, which might account for the difference reported by Moss et al. (1997). Our methodology allowed us to control for multiple variables simultaneously, including the most important factor influencing snakebite severity—snake size—which corresponds to the mass of venom injected.

How quickly a rattlesnake bite victim gets medical care after the bite may also influence bite severity, with longer lag times tending to result in greater clinical severity (Silveira & De Andrade Nishioka 1992; Pinho et al. 2005). However, consistent other studies (Bucaretchi et al. 2002; Gerardo et al. 2015), our results did not show this relationship. This may be because the vast majority of patients in our study received medical care very quickly. Half of our patients arrived at a medical facility within 1 hr, and 90% arrived within 3 hr. This may have rendered the relationship undetectable. The only other study that has examined time to treatment for CroFab (Gerardo et al. 2015) failed to detect an effect on antivenom use when comparing time to antivenom treatment of greater than 6 hours to less than six hours. However, this study may not be directly comparable since the majority of cases were Copperhead envenomations, which generally give less severe bites. The effects of increased time until treatment may also result in long-term or permanent tissue damage that can only be assessed after initial discharge from the hospital—a timeframe outside the scope of this study.

Limitations

Despite our attempt to maintain the highest possible methodological rigor, several possible shortcomings are common to studies based on retrospective chart reviews. First, bias may exist in the type and extent of missing data. More complete data may have been collected for patients with more severe bites because these cases were likely to have received more attention from a greater number of clinicians, including a well-informed snakebite specialist (SPB). Also, charts did not specify whether recorded patient mass was measured or estimated. Since it may be more difficult to measure the mass of patients confined to a bed, a greater proportion of estimated masses may have been recorded for patients with more severe envenomations. Second, possible error existed in the information we used to determine the size and species of the snake. For information on the size and species of the snake, determinations based on photographs, specimens brought to the hospital, or measurement of fang-spread, especially those evaluated by SPB, were considered to be of high accuracy. However, we also based determinations on descriptions provided by other medical personnel (e.g., first responders) and the patients themselves, and these were likely to be less accurate. Cross-referencing the geographic location where the bite occurred with the known geographic ranges of southern California rattlesnakes provided some error mitigation for species determinations. Third, veracity of the patients comprised another potential source of error, particularly with respect to assigning bite legitimacy. Patients may have felt ashamed if their own lack of judgement had resulted in the bite, or may have worried about legal ramifications, especially if they were keeping the snake in captivity, and therefore may have given inaccurate information about how the bite occurred. In one case where a male patient was transferred to LLUMC from another medical facility, we found contradictory information surrounding the circumstances of the bite. This patient reportedly told LLUMC medical personnel that a snake had come out of the bushes and bit him on the finger while he was sitting outside at a friend’s house. However, the story reported by the outside facility was that he was bitten on the finger by a rattlesnake his friend was keeping as a "pet." Such reluctance to admit to an illegitimate bite suggests that our finding that 45.8% of classifiable cases were illegitimate may be an underestimate.

Conclusions

Our results support several conclusions that may be of use by clinicians treating venomous snakebite in southern California. First, most snakebites occur during the spring, but another large group of snakebites occur in the fall, primarily from a pulse of newborn snakes. Clinicians and pharmacologists should be aware of this, and prepare accordingly. Second, envenomations delivered by larger snakes and to smaller patients tend to be more severe. Clinicians should therefore treat such cases more aggressively, and should seek information about the size of the snake upon initial presentation of a patient to a medical facility. Third, CroFab is efficacious against all southern California rattlesnake venoms, including that of *C. o. helleri*. When controlling for snake size and patient body mass, we found no significant differences in CroFab effectiveness among species. However, recent study suggests that the clinical syndrome can vary substantially among snake species (Appendix 1), and therefore ascertainment of offending snake species may be valuable to anticipating and managing the course of treatment.

Our results further suggest that at least 45.8% of venomous snakebites in southern California are illegitimate, and therefore preventable. Males and intoxicated individuals are at greatest risk of sustaining bites. Education initiatives that promote leaving the snake alone may help to reduce the number of snakebites in southern California and elsewhere.

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CHAPTER FOUR

CLINICAL PREDICTION OF OVERALL SEVERITY, SYMPTOM PROGRESSION, AND ANTIVENOM USE IN VICTIMS OF VENOMOUS SNAKEBITE IN SOUTHERN CALIFORNIA

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Abstract

Despite low mortality in the U.S., venomous snakebite constitutes a potentially life-threatening medical emergency that often results in a high financial cost to victims in large part due to the cost of antivenom (CroFab). To improve decisions by physicians treating snake envenomation in southern California, we assessed several factors currently omitted from snakebite treatment algorithms for their potential usefulness as predictors of maximal snakebite severity score (mSSS, based on the sum of six clinical symptom subscores, SSsS), symptom progression from initial assessment, and antivenom use. The factors included initial SSS (iSSS), size of the envenoming snake, mass of the patient, snake species, anatomical location of bite, whether the bite was provoked (illegitimate) or not (legitimate), and the time until hospital admission. We found initial snakebite severity score (iSSS), the size of the envenoming snake, and patient mass to be significant predictors of overall bite severity, symptom progression, and antivenom use. Initial SSS proved to be the most effective predictor of overall severity, explaining ~70% of the variance. Snake size best predicted symptom progression, with larger snakes inflicting greater symptom progression. Patient mass and iSSS also significantly predicted symptom progression, with smaller patients and those with lower iSSSs experiencing greater symptom progression. Snake size was also the most important factor predicting antivenom use, with large snakes requiring, on average, seven more vials of CroFab than medium or small snakes. We further evaluated whether each of the six symptoms upon admission (iSSS subscores for cardiovascular, gastric, hematological, local wound, neurological, and pulmonary symptoms) influenced overall SSS, symptom progression, and antivenom use. All six subscores significantly predicted overall severity, but hematological, neurological, and cardiovascular subscores were most salient. Local wound score was the only significant predictor of symptom progression. Gastric, neurological, and hematological symptoms significantly influenced antivenom use, but the gastric subscore showed an unexpected inverse relationship to antivenom use. Based on our analyses, we suggest that iSSS, snake size, and patient mass are especially useful to clinicians for anticipating antivenom needs. We further suggest several rules of thumb that could be added to the current snakebite treatment algorithm to help clinicians anticipate antivenom needs.

Introduction

Several thousand human envenomations from venomous snakes occur in the United States every year (estimated at 2,683–3,858; Kasturiratne et al. 2008), with the vast majority of these being caused by pitvipers (family Viperidae, subfamily Crotalinae; Seifert et al. 2009). Despite a low mortality rate (5–7 deaths annually; Kasturiratne et al., 2008), venomous snakebite in the United States constitutes a potentially life-threatening medical emergency. Non-fatal envenomations can still result in significant hematotoxicity, neurotoxicity, and local soft tissue damage, and can lead to long-term physical and emotional morbidity (Dart et al. 1992; Smith & Bush 2010; Williams et al. 2011).The financial costs to victims of snakebite can also be high. The most definitive treatment for snake envenomation remains antivenom infusion (Smith & Bush 2010). Currently, Crotalidae Polyvalent Immune Fab (Ovine) (CroFab®; Protherics, Brentwood, TN, USA, now part of BTG International, London, UK) is the only FDA-approved antivenom to treat pitviper bites in the U.S., and wholesales at more than $1000 per vial (Corneille et al. 2006; Lavonas et al. 2011). Given the average of 14 vials of CroFab needed to resolve envenomations in southern California (Chapter 3), the cost to patients or insurers from antivenom alone can add up very quickly. With a more than two-fold mark-up in cost, antivenom may comprise more than 70% of the total hospital bill (Corneille et al. 2006). Cases wherein hospitals have billed patients $20,000 or more per vial have been reported in the popular media (Marcinko 2014; Rhodan 2014). The high rate of symptom recurrence (Boyer et al. 1999; Boyer et al. 2001; O'Brien et al. 2009; Lavonas et al. 2014) and low rate of hypersensitivity reactions (Dart & McNally 2001; Cannon et al. 2008) also drive a tendency toward overuse, which may exacerbate patient costs. Indeed, one study has documented a significant increase in the use of antivenom since CroFab was introduced in 2001 (Spiller et al. 2010), and Lavonas et al. (2011) noted that inexperienced health care providers may administer large doses to treat clinical effects that do not respond to antivenom therapy, and could be safely observed without further treatment.

The need to maximize patient benefits while reducing the risks and costs of antivenom use requires the standardization of care via an evidence-based treatment algorithm. Such an algorithm has been developed for the treatment of pit viper bites in the United States (Lavonas et al. 2011). However, this algorithm does not make use of grading scales for assessing the severity of crotaline envenomations, preferring instead to make use of continuous assessment of specific venom effects in order to inform treatment decisions. This approach was taken because the validity, reliability, and utility of such grading scales have yet to be demonstrated in a clinical setting. However, such grading scales, coupled with other baseline information obtained during initial clinical assessment, could prove useful if they could help predict overall severity and determine which patients were most likely to show symptom progression. This information, therefore, could help medical professionals anticipate complications and improve patient outcomes.

Several key factors affect bite severity and the clinical symptoms observed; these include the size and species of snake, as well as the body size and general health of the patient (Hayes & Mackessy 2010). Recent research suggests that the most important of these factors are snake size and patient mass (Chapter 3). Although snake species often differ in venom composition, their bites generally elicit different symptoms but result in similar overall severity (Chapter 3 and Appendix 1; Hayes et al. 2005; Janes et al. 2010). However, much of the research exploring these relationships has been general in nature, and has not focused on specific clinical application.

The aim of this research was to examine the potential clinical usefulness of a standardized snake envenomation grading scale, the snakebite severity score (SSS; Dart, Hurlbut, Garcia, & Boren, 1996). In particular, we sought to determine whether the assessment of SSS and other relevant factors at the initial presentation of a snakebite victim at the hospital could help predict (1) the overall severity of envenomation, (2) the progression of symptoms following initial presentation, and (3) the total vials of CroFab antivenom used in treatment.

Materials and Methods

Study Design

We analyzed data retrospectively abstracted from 243 medical records of venomous snakebite victims admitted between 2001 and 2010 to the Emergency Department of the Loma Linda University Medical Center (LLUMC). Our dataset was a subset of a larger dataset of 354 cases (see Chapter 3), but included only those patients treated exclusively with CroFab. For the larger, original dataset, cases were identified by a database search for records between 1990 and 2010 that contained the International Classification of Diseases, Ninth Revision (ICD-9), codes E905.0 (venomous snake and lizard bites) and 989.5 (toxic effect of venom). Some records lacking these codes were undoubtedly missed. We included patients if they were bitten by a venomous snake native to southern California. We excluded patients that were bitten by animals other than venomous snakes, those bitten by venomous species not native to Southern California, and those whose envenomation did not result from a bite (one patient had an eye splashed by venom). For each patient, data were collected that covered the period of time between the bite and initial discharge. Information from follow-up visits was not considered. The protocol was reviewed by the institutional review board and considered exempt from informed consent.

Data Collection

Abstractors included one of the investigators (AGC) and four research assistants, none of whom were blinded to the goals of the study. The four research assistants were trained in data collection and calculation of snakebite severity by one of the investigators (AGC) via use of a standardized abstraction form. Inter-rater reliability was assessed and showed substantial agreement between abstractors (see Chapter 3).

Calculating Snakebite Severity Scores

We calculated snakebite severity scores (SSS) using the rubric designed by Dart et al. (1996). This scoring method, which ranges from 0–20 points, is based on the objective evaluation of clinical parameters in six categories or subscores (SSsS): local wound effects, hematologic (coagulation) parameters, and symptoms associated with the pulmonary, cardiovascular, gastrointestinal, and central nervous systems. The subscores for each of these categories, which ranged from 0–3 or 0–4, were recorded separately and then summed to obtain a final SSS. Higher SSS scores indicate a more severe bite. If the patient record included no mention of an organ system abnormality relevant to any category, then that system was assumed to be unaffected by the snakebite. Because the SSS criteria published by Dart et al. (1996) were designed to assess adult patients, we adjusted the scoring of pulmonary and cardiac categories to account for differences in respiratory rate, heart rate, and blood pressure between children and adults (see Chapter 3). These adjustments were based on other pediatric medical assessment rubrics and published data on normal values for pediatric vital signs (Tepas et al. 1987; Pollack et al. 1997; Fleming et al. 2011; U.S. Department of Health & Human Services 2011) in consultation with a pediatric emergency medicine specialist.

We calculated two separate SSS scores for each case. Initial SSS (iSSS) was calculated by determining the maximum scores for each category based on information recorded from time of the bite until the patient received their first dose of antivenom. Maximal SSS (mSSS) was determined by taking the maximum scores for each category based on information recorded from the time of the bite until initial discharge from the hospital. To assess the progression of snakebite symptoms once victims were in the care of medical professionals, we calculated the increase in SSS (incSSS) by subtracting iSSS from mSSS.

Determining Size of the Snake

For 186 (76.5%) of the 243 cases used for this analysis, we categorized snake size as small (< 40 cm snout-vent length), medium (40–75 cm), or large (>75 cm), following the methods of Chapter 3. Briefly, we relied on three approaches for judging body size: 1) the treating physician measured the length of the snake if it was brought to the hospital with the patient; 2) we used a regression model to estimate snake length based on the space between fang puncture wounds; and 3) we recorded a qualitative size assessment (e.g., "baby," "small," "large") from observers deemed reliable.

Determining Species of Snake

Seven taxa of rattlesnakes occur in southern California, including *Crotalus atrox*, *C. cerastes*, *C. mitchellii pyrrhus*, *C. oreganus helleri*, *C. oreganus oreganus*, *C. ruber ruber*, and *C. scutulatus scutulatus*. Because *C. oreganus* is represented by two subspecies in this region, we use the generic term "snake species" without implying anything about the taxonomic relationship within this clade. Of the 243 cases analyzed in this study, we were able to determine the envenoming species in 152 (62.6%) of them. Species were assigned to cases as described in Chapter 3. To summarize, the majority of species identifications were made by SPB based on a specimen or photograph brought to the hospital with the patient. Otherwise, species assignment was based on detailed descriptions when provided by reliable observers, as recorded in the medical record. Species assignments were checked against the geographical range and preferred habitat of each snake species (Stebbins 2003; Campbell & Lamar 2004).

Other Variables

We analyzed several additional variables extracted from each record, including the number of vials of CroFab administered, patient mass, time to hospital admission (elapsed hours between bite and admission to hospital), limb bitten (upper or lower extremity), site of bite (distal or proximal to the wrist or ankle), and the type of interaction the patient had with the snake. Following the definitions provided by Klauber (1956) and Russell (1983), we classified the interactions as “legitimate” or “illegitimate.” If the patient saw the snake, and his/her deliberate interaction with the snake caused the bite, then the interaction was deemed “illegitimate.” If the interaction was not deliberate, and the patient did not see the snake prior to the bite, then the interaction was classed as “legitimate.”

Statistical Analyses

We conducted statistical analyses using SPSS 13.0 (SPSS Inc., Chicago, IL) and R version 3.1.2 (R Core Team 2014). All hypothesis testing was done using SPSS 13.0 with standard defaults and alpha set at 0.05, whereas R version 3.1.2 was used to calculate Cook’s *D* for all analyses involving linear models (see below). Following Nakagawa (2004), we chose not adjust alpha for multiple tests. Unless otherwise indicated, we report values as mean ± 1 S.E.

Factors Predicting Maximal Severity

We relied on five analysis of covariance (ANCOVA) models (Mertler & Vannatta 2004) and post-hoc multiple comparisons (least significant difference, LSD) to determine which of several factors affected mSSS as the dependent variable. We had to use multiple models because a single omnibus model that included all independent variables of interest resulted in too many empty cells, as many cases lacked information on snake species, snake size, and/or type of patient interaction with the snake. For each of these models, we used mSSS as the dependent variable. Based on prior research (Wingert & Chan 1988; Blaylock 2004; Hayes et al. 2005; Benítez et al. 2007; Janes et al. 2010; Chapter 3 of this dissertation), we controlled for two primary predictors in all five models: snake size (as a fixed factor) and patient mass (as a covariate). We also included iSSS (covariate) in all models as a third primary predictor. The remaining five secondary predictors entered into the models included: snake species, limb bitten (upper or lower), site of bite (proximity to wrist or ankle), interaction with snake (legitimate or illegitimate), and time to hospital admission, with the first four variables treated as fixed factors, and the latter as a covariate. To maximize sample size and statistical power, each of the secondary predictors was entered into a separate model with the three primary predictors, except that one model included only the primary predictors, and two predictors (limb bitten and site of bite) were included together in another single model. Since the data largely conformed to parametric assumptions, none of the variables were transformed. For each model, we identified outliers using Cook's *D* (Cook 1977), with cases being omitted as outliers if *D* exceeded 4/n (Bollen & Jackman 1990).

We subsequently used a sixth ANCOVA model to analyze the contribution of each iSSS subscore (local wound effects, hematologic = coagulation parameters, and symptoms associated with the pulmonary, cardiovascular, gastrointestinal, and central nervous systems) to mSSS while controlling for snake size and patient mass, as indicated by results of the first five ANCOVA models. This analysis allowed us to infer which initial symptoms were most predictive of overall snakebite severity.

Factors Predicting Symptom Progression

To assess the factors that predict the relative symptom progression following assessment of iSSS, we utilized another five ANCOVA models, making use of the same factors and covariates as for the previous analyses. However, we used incSSS as the dependent variable rather than mSSS, and we rank-transformed both incSSS and iSSS to better meet parametric assumptions. As with mSSS, we also utilized a sixth ANCOVA model to analyze the contribution of each iSSS subscore to relative symptom progression. Based on the results of the first five ANCOVA models, this model also included snake size and patient mass.

Progression of Specific Symptom Types

To assess which specific snakebite severity subscores (SSsS) showed progression in patients during the course of treatment, we compared initial and maximal values for each SSsS. Progression was deemed present if there was a difference. Cochran's Q (Cochran 1950) was used to test for differences in the proportion of cases that showed progression among the six subscores.

Factors Predicting Amount of Antivenom Used

To assess the factors that predict the amount of antivenom used, we utilized another five ANCOVA models making use of the same factors and covariates as for the previous analyses. However, the total number of vials of antivenom was used as the dependent variable instead of mSSS or incSSS. For these analyses, we did not transform vials of antivenom or iSSS so as to make the analysis more directly applicable for clinical application and because the data largely conformed to parametric assumptions after omitting outliers utilizing the aforementioned criteria. As with mSSS and incSSS, we also utilized a sixth ANCOVA model to analyze the contribution of each iSSS subscore to the amount of antivenom used. Again, this model also included snake size and patient mass.

Assumptions and Effect Sizes

For all ANCOVA models, we tested the assumption of homogeneity of regression slopes using separate ANCOVA models that included all interactions between the covariates and other predictors. No significant interactions were found, so we omitted interactions involving the covariates from our final models. We computed the coefficients (β) of the underlying regression model for each covariate to aid interpretation. We computed effect sizes as partial eta-squared (η^2), with values of ~0.01, ~0.06, and >0.14 loosely regarded as small, medium, and large effects, respectively (Cohen 1988). Although partial η^2 values become upward biased as more independent variables are added to the model (Pierce et al. 2004), they never summed to >1 in the models tested, and therefore were not adjusted.

Results

Factors Predicting Maximal Snakebite Severity

Mean mSSS for all cases was 7.12 ± 0.22. Of the eight independent variables and cofactors used among the five ANCOVA models (snake size, snake species, iSSS, patient mass, limb bitten, site of bite, interaction with snake, time to hospital admission), only snake size (all $P \leq 0.031$; partial $\eta^2 = 0.07$–0.13), patient mass (all $P \leq 0.001$; partial $\eta^2 =$

0.10–0.15), and iSSS (all $P < 0.001$; partial $\eta^2 = 0.67–0.70$) were significant, and these were significant in all five models (Table 1). Estimated marginal means for mSSS were calculated for each snake size class based on Model 1, and were 6.66 ± 0.17, 6.69 ± 0.24, and 8.11 ± 0.25 for small, medium, and large snakes, respectively. Post-hoc (LSD) comparisons for model 1 (the model containing only the significant independent variables) showed that bites from large snakes had higher mSSSs than those from small and medium snakes ($P < 0.001$ for both), whereas the mSSS for bites of small and medium snakes was similar ($P = 0.916$). Initial SSS was negatively associated with patient mass (β = -0.02 to -0.03) and positively associated with iSSS (β = 0.79–0.89).

The sixth ANCOVA model examining the effects of each iSSS subscore on mSSS is reported in Table 2. All six subscores (cardiovascular, gastric, hematological, pulmonary, neurological, and local wound) were found to be significant predictors of mSSS, with the regression coefficients suggesting a direct relationship for each subscore. Effect sizes (partial η^2) suggested the following ranking for each subscore's impact on mSSS: hematological > neurological > cardiovascular > pulmonary > gastric > local wound.

Table 1. Results (*P*-values and partial η^2 effect sizes) of analysis of covariance (ANCOVA) models for maximal evenomation severity (mSSS) resulting from bites from seven rattlesnake species in southern California.

	Model 1 (*N* = 178)		**Model 2 (*N* = 117)**[a]		**Model 3 (*N* = 171)**[a]		**Model 4 (*N* = 159)**		**Model 5 (*N* = 174)**	
Independent variables	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**
Snake size	**<0.001**	0.13	**0.031**	0.07	**0.001**	0.08	**<0.001**	0.10	**<0.001**	0.13
Patient mass	**<0.001**	0.15	**0.001**	0.10	**<0.001**	0.10	**<0.001**	0.15	**<0.001**	0.11
iSSS	**<0.001**	0.70	**<0.001**	0.70	**<0.001**	0.67	**0.001**	0.70	**<0.001**	0.69
Snake species	–	–	0.648	0.03	–	–	–	–	–	–
Snake species × snake size	–	–	0.303	0.08	–	–	–	–	–	–
Upper vs. lower limb	–	–	–	–	0.982	<0.01	–	–	–	–
Proximal vs. distal bite	–	–	–	–	0.242	0.01	–	–	–	–
Proximal or distal bite × snake size	–	–	–	–	0.591	<0.01	–	–	–	–
Upper or lower limb × snake size	–	–	–	–	0.887	<0.01	–	–	–	–
Proximal or distal × upper or lower limb	–	–	–	–	0.099	0.02	–	–	–	–
Proximal or distal × upper or lower limb × snake size	–	–	–	–	0.999	<0.01	–	–	–	–
Interact with snake	–	–	–	–	–	–	0.664	0.00	–	–
Interact × snake size	–	–	–	–	–	–	0.182	0.02	–	–
Time to hospital admission	–	–	–	–	–	–	–	–	0.881	<0.01

[a]Type IV sum of squares used due to empty cells

Table 2. Results of ANOVA model with maximal snakebite severity scores (mSSS) as the dependent variable, and each SSS subscore (shown), rank of patient mass (not shown), and size of snake (not shown) treated as independent factors. Regression coefficients (*β*) and effect sizes (partial η^2) are also included. Subscores are ordered by effect size.

SSS Subscore	$F_{1,156}$	***P***	***β***	**Partial** η^2
Hematological	57.07	<0.001	0.95	0.27
Neurological	39.80	<0.001	0.96	0.20
Cardiovasular	26.92	<0.001	0.95	0.15
Pulmonary	13.72	<0.001	0.78	0.08
Gastric	9.37	0.003	0.68	0.06
Local Wound	5.62	0.019	0.39	0.04

Factors Predicting Symptom Progression

Mean incSSS was 2.40 ± 0.133. Of the eight independent variables and cofactors used among the five ANCOVA models (snake size, snake species, iSSS, patient mass, limb bitten, site of bite, interaction with snake, time to hospital admission), only snake size (all $P \leq 0.010$; partial η^2 = 0.06–0.16), patient mass (all $P \leq 0.001$; partial η^2 = 0.10–0.14), and iSSS (all $P \leq 0.008$; partial η^2 = 0.07–0.11) were significant, and these were significant in all five models (Table 3). Post-hoc analysis of model 1 (the model containing only the significant independent variables) showed that bites from large snakes had more symptom progression than bites from both small and medium snakes ($P < 0.001$ for both), whereas progression of symptoms from the bites of small and medium snakes was similar ($P = 0.512$). Increase in SSS was negatively associated with both patient mass (β = -0.72 to -0.99) and iSSS (β = -0.18 to -0.21).The sixth ANCOVA model examining the effects of each iSSS subscore on incSSS is reported in Table 4. Of the six subscores (cardiovascular, gastric, hematological, pulmonary, neurological, and local wound), only the local wound subscore was found to significantly predict incSSS. The regression coefficient (β = -26.23) suggested an inverse relationship between this subscore and incSSS; in other words, higher initial local wound subscores predicted less progression of symptoms.

Progression of Specific Symptom Types

Some snakebite severity subscores were more likely than others to show progression of symptoms (Cochran's Q = 180.90, $df = 5$, $p < 0.001$; Figure. 1). The proportion of cases in which symptom progression occurred showed the following rankings for each symptom category: local wound > hematological > cardiovascular > pulmonary > neurological > gastric.

Factors Predicting Antivenom Usage

Median vials of CroFab used for all cases was 12.0 (range 2–66). Of the eight independent variables and cofactors used among the five ANCOVA models (snake size, snake species, patient mass, limb bitten, site of bite, interaction with snake, time to hospital admission), the main effects of the primary predictors snake size, patient mass, and iSSS were significant for all models except model 2 (Table 5; for models 1, 3 ,4 and 5, snake size: all $P < 0.001$, partial $\eta^2 = 0.11–0.20$; patient mass: all $P \leq 0.019$, partial $\eta^2 = 0.03–0.08$; iSSS: all $P \leq 0.024$, partial $\eta^2 = 0.03–0.06$). Estimated marginal means for total vials of antivenom were calculated for each snake size class based on Model 1, and were 12.14 ± 0.61, 11.67 ± 0.89, and 18.42 ± 0.95 for small, medium, and large snakes, respectively. Post-hoc pair-wise comparisons showed significant differences between large snakes and both medium and small snakes ($P < 0.001$ for both), whereas small and medium snakes were similar in antivenom dosage ($P = 0.676$).

Table 3. Results (*P*-values and partial η² effect sizes) of analysis of covariance (ANCOVA) models for increase in envenomation severity (incSSS) between initial (iSSS) and maximal (mSSS) measures of snakebite severity resulting from bites from seven rattlesnake taxa in southern California.

	Model 1 (*N* = 178)		**Model 2 (*N* = 117)**[a]		**Model 3 (*N* = 171)**[a]		**Model 4 (*N* = 159)**		**Model 5 (*N* = 174)**	
Independent variables	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**	***P***	**η^2**
Snake size	**<0.001**	0.16	**0.010**	0.09	**0.003**	0.06	**<0.001**	0.12	**<0.001**	0.12
Patient mass	**<0.001**	0.14	**<0.001**	0.11	**<0.001**	0.10	**<0.001**	0.14	**<0.001**	0.12
iSSS	**<0.001**	0.08	**0.008**	0.07	**<0.001**	0.11	**0.001**	0.07	**<0.001**	0.07
Snake species	–	–	0.847	0.02	–	–	–	–	–	–
Snake species × snake size	–	–	0.113	0.10	–	–	–	–	–	–
Upper vs. lower limb	–	–	–	–	0.649	<0.01	–	–	–	–
Proximal vs. distal bite	–	–	–	–	0.945	<0.01	–	–	–	–
Proximal or distal bite × snake size	–	–	–	–	0.947	<0.01	–	–	–	–
Upper or lower limb × snake size	–	–	–	–	0.734	<0.01	–	–	–	–
Proximal or distal × upper or lower limb	–	–	–	–	0.153	0.01	–	–	–	–
Proximal or distal × upper or lower limb × snake size	–	–	–	–	0.999	<0.01	–	–	–	–
Interact with snake	–	–	–	–	–	–	0.780	0.00	–	–
Interact × snake size	–	–	–	–	–	–	0.438	0.01	–	–
Time to hospital admission	–	–	–	–	–	–	–	–	0.517	<0.01

Independent variables: Patient age, patient mass, and time to hospital all rank transformed and treated as covariates. All others treated as fixed factors.
[a]Type IV sum of squares used due to one empty cell

Table 4. Results of ANOVA model with rank of increase in snakebite severity scores (incSSS) as the dependent variable, and each SSS subscore (shown), rank of patient mass (not shown), and size of snake (not shown) treated as independent factors. Regression coefficients (β) and effect sizes (partial η^2) are also included. Subscores are ordered by effect size.

SSS Subscore	$F_{1,157}$	P	β	Partial η^2
Local Wound	18.82	<0.001	-26.23	0.10
Hematological	0.98	0.324	-4.56	0.01
Gastric	0.66	0.420	-6.68	<0.01
Pulmonary	0.51	0.477	-5.55	<0.01
Cardiovasular	0.31	0.578	-3.97	<0.01
Neurological	0.12	0.735	1.89	<0.01

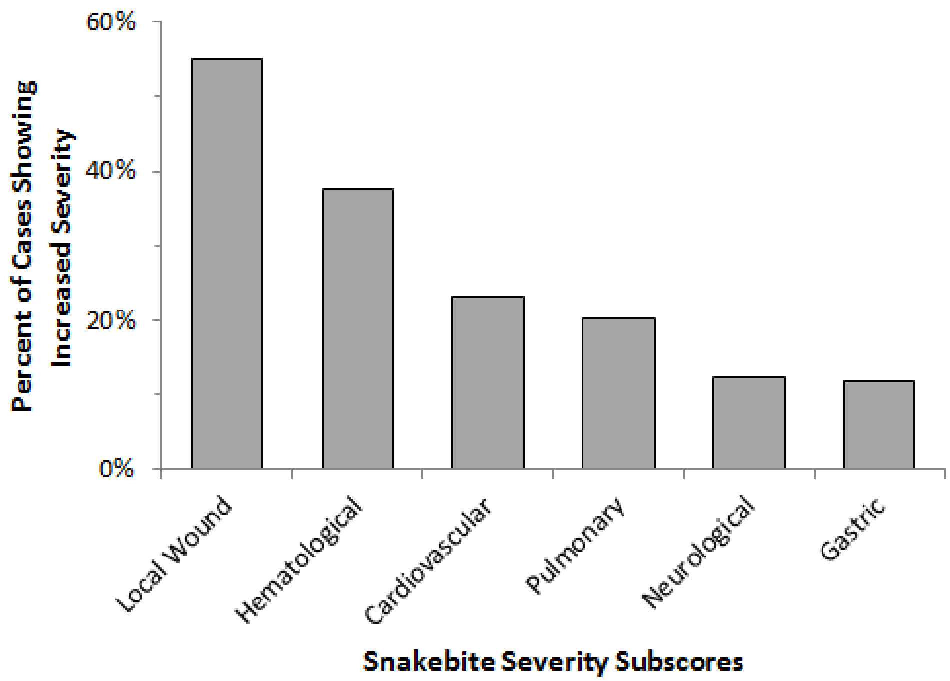

Figure 1: Percent of snake bite cases (out of 243) showing increases in snakebite severity between initial presentation and maximal severity for the six snakebite severity subscores corresponding to specific clinical symptoms.

Table 5. Results (*P*-values and partial η^2 effect sizes) of analysis of covariance (ANCOVA) models for antivenom usage resulting from rattlesnake bites in southern California

Independent variables	**Model 1 (*N* = 170)**		**Model 2 (*N* = 117)[a]**		**Model 3 (*N* = 168)**		**Model 4 (*N* = 155)**		**Model 5 (*N* = 168)**	
	P	η^2	***P***	η^2	***P***	η^2	***P***	η^2	***P***	η^2
Snake size	**<0.001**	0.18	0.361	0.02	**<0.001**	0.20	**<0.001**	0.11	**<0.001**	0.16
Patient mass	**0.019**	0.03	0.077	0.03	**0.009**	0.04	**0.001**	0.08	**0.015**	0.04
iSSS	**0.021**	0.03	0.055	0.04	**0.001**	0.06	**0.024**	0.03	**0.007**	0.04
Snake species	–	–	0.093	0.09	–	–	–	–	–	–
Snake species × snake size	–	–	0.122	0.11	–	–	–	–	–	–
Upper vs. lower limb	–	–	–	–	0.417	<0.01	–	–	–	–
Proximal vs. distal bite	–	–	–	–	0.739	<0.01	–	–	–	–
Proximal or distal bite × snake size	–	–	–	–	0.863	<0.01	–	–	–	–
Upper or lower limb × snake size	–	–	–	–	**0.001**	0.09	–	–	–	–
Proximal or distal × upper or lower limb	–	–	–	–	0.952	<0.01	–	–	–	–
Proximal or distal × upper or lower limb × snake size	–	–	–	–	0.999	<0.01	–	–	–	–
Interact with snake	–	–	–	–	–	–	0.969	<0.01	–	–
Interact × snake size	–	–	–	–	–	–	**0.008**	0.06	–	–
Time to hospital admission	–	–	–	–	–	–	–	–	0.990	<0.01

Independent variables: Patient age, patient mass, and time to hospital all rank transformed and treated as covariates; all others treated as fixed factors.

[a]Type IV sum of squares used due to one empty cell

Number of vials of CroFab used was negatively associated with patient mass (β = -0.04 to -0.07) and positively associated with iSSS (β = 0.42–0.54

None of the main effects of the secondary predictors (snake species, upper versus lower limb, proximal versus distal bite, interaction with the snake, time to hospital admission) were significant in these models. However, a significant interaction existed between snake size and upper vs. lower limb ($F_{1,156}$ = 7.35, P = 0.001, partial η^2 = 0.09) in model 3. An interaction plot (Figure 2A) suggested that patients with bites from large snakes to the lower extremity were given more antivenom than similar bites to the upper limb, whereas similar quantities of antivenom were given regardless of site of bite for small and medium snakes. Model 4 also revealed a significant interaction between snake size and whether the bite was legitimate or illegitimate ($F_{2,147}$ = 5.00, P = 0.008, partial η^2 = 0.06). An interaction plot (Figure 4) suggested that patients who sustained legitimate bites from large snakes were given more antivenom on average than those receiving illegitimate bites, but this pattern was reversed for medium snakes.

The sixth ANCOVA model examining the effects of each iSSS subscore on antivenom usage is reported in Table 6. Gastric, neurological, and hematological subscores were significant predictors of antivenom administration (in this order; $P \leq$ 0.044, partial η^2 = 0.03–0.06), whereas local wound, cardiovascular, and pulmonary subscores were not. Regression coefficients suggested an inverse relationship between the gastric subscore and antivenom use (β = -2.77), and a direct relationship for the neurological and hematological scores (β = 1.33 and 1.06, respectively). Effect sizes (partial η^2) suggested the following ranking for each significant subscore's impact on antivenom use: gastric > neurological > hematological.

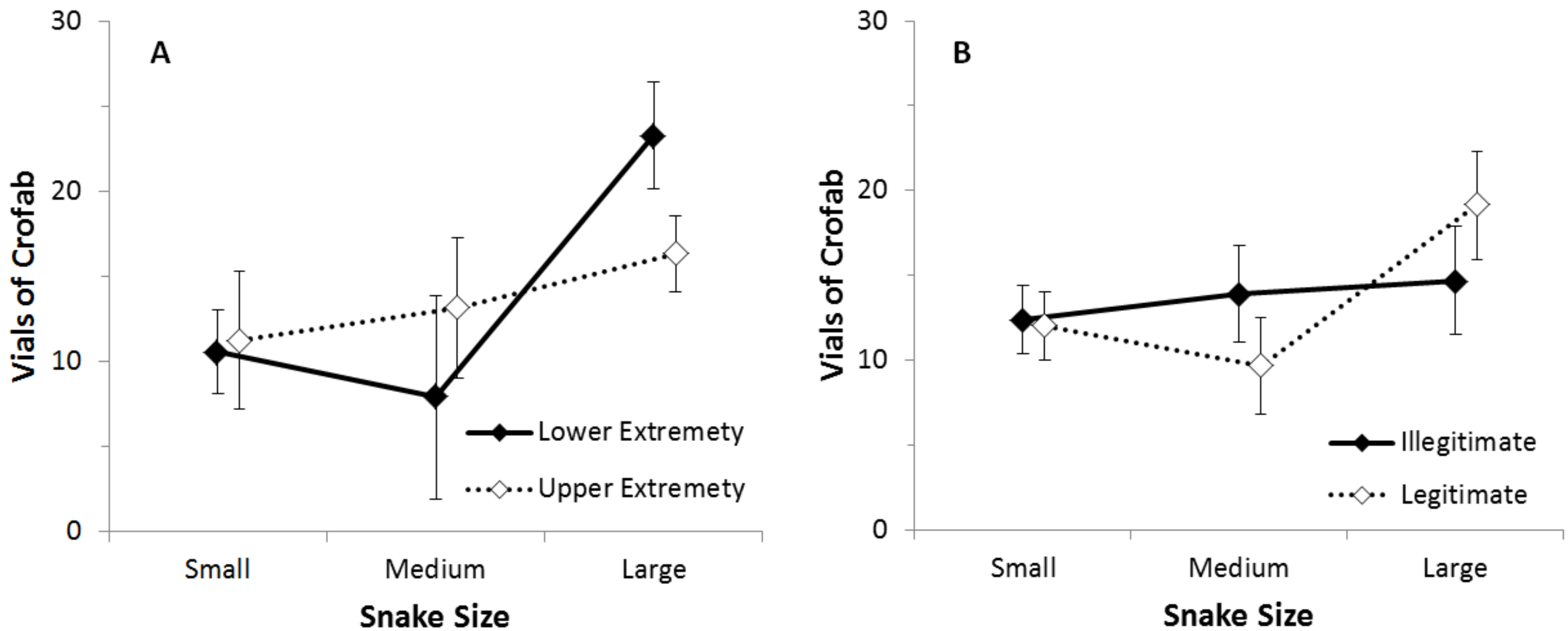

Figure 2: Interaction plots showing the effect of snake size (small: < 40 cm; medium: 40–75 cm; and large: >75 cm) and (A) whether the bite was to the upper vs. lower extremity, or (B) whether the bite was provoked (illegitimate) or not (legitimate), on the estimated marginal means of CroFab vials needed to treat the envenomation. Panel A is based on model 3 and panel B is based on model 4 from Table 5. Patient mass and initial snakebite severity (iSSS) were held constant (Panel A: 69.35 kg and 4.51, respectively; Panel B: 75.79 kg and 4.77, respectively). Error bars represent 95% confidence intervals.

Table 6. Results of ANOVA model that with vials of CroFab as the dependent variable, and each initial subscore (shown), rank of patient mass (not shown), and size of snake (not shown) as independent variables. Regression coefficients (β) and effect sizes (partial η^2) are also included. Subscores are ordered by effect size.

SSS Subscores	$F_{1,154}$	*P*	β	Partial η^2
Gastric	9.14	0.003	-2.77	0.06
Neurological	5.08	0.026	1.33	0.03
Hematological	4.13	0.044	1.06	0.03
Local Wound	2.01	0.158	0.89	0.01
Cardiovascular	1.95	0.165	0.993	0.01
Pulmonary	1.28	0.259	-0.961	0.01

Discussion

In this study, we sought to identify the salient factors that could help a physician anticipate how severe a snakebite might become subsequent to the initial presentation. Based on a substantial clinical data set and several measures of the well-established SSS rubric, we used multivariate models to evaluate the potential predictors of 1) maximal snakebite severity, 2) specific symptom profession, and 3) antivenom usage. Based on our findings, we offer several recommendations for treating physicians.

Factors Predicting Maximal Severity

We found that snake size and patient mass were significant predictors of overall snakebite severity, with each generally having a moderate to large effect size. Our analyses showed that large snakes were responsible for greater overall envenomation severity than medium or small snakes, which is consistent with previous research (Hayes et al. 2005; Hayes & Mackessy 2010; Janes et al. 2010); Chapter 3 of this dissertation). Also consistent with previous research is the finding of an inverse relationship between patient mass and mSSS (Hayes et al., 2005; Hayes & Mackessy, 2010; Chapter 3 this dissertation). We further found iSSS to be a very significant predictor that showed a strong positive relationship to mSSS, explaining ~70% of the variance (partial η^2 value; Table 1) This very large effect size may be explained by the fact that a majority of our cases didn't show a significant increase in symptomology beyond initial assessment. Indeed, median increase in severity was just two SSS points, and 90.1% of cases showed an increase of just five or less. These findings suggest that the majority of the envenomation severity is captured by iSSS, and that substantial progression of symptoms beyond those seen at the initial assessment may be relatively rare.

All iSSS subscores were significant predictors of mSSS. However, the hematological, neurological, and cardiovascular subscores showed large effect sizes, whereas the pulmonary, gastric, and local wound subcores showed small to moderate effect sizes. This finding is consistent with Yin et al., (2011) who found that thrombocytopenia, bleeding, neurologic effects, and bite severity were associated with difficulty in achieving initial control with antivenom administration.

Factors Predicting Symptom Progression

Our results suggest that the major factors that predict the progression of symptoms beyond the initial clinical assessment are the size of the envenoming snake, the mass of the patient, and the iSSS. Of these factors, the one with the greatest predictive value was the size of the snake, which in our analysis explained ~16% of the variance (Table 2), with larger snakes more likely to cause greater symptom progression. Patient mass explained ~14% of the variance, with smaller patients showing significantly greater symptom progression. Surprisingly, our statistical models suggested an inverse relationship between iSSS and severity progression, with higher iSSS scores predicting a reduction in the amount of symptom progression.

In analyzing the prediction of symptom progression based on iSSS subscores, only the local wound subscore was significant, explaining ~10% of the variance. The other five subscores (hematological, gastric, pulmonary, cardiovascular, and neurological) were not significant. This result may be explained by differences in the rate at which various symptoms progress. The symptoms associated with the local wound subscore generally progress more slowly, causing this subscore to reach peak severity much later than the other subscores. This finding further suggests that the majority of symptom progression after iSSS assessment may be due to progression of local wound symptoms.

Our analyses found no evidence that the anatomical location of the bite or the nature of interaction with the snake (legitimate or illegitimate) affected symptom progression. Our analyses further found no evidence that symptom progression varied among snake species. This latter result was somewhat unexpected considering the differences in venom composition and clinical symptoms among the seven venomous snake taxa in southern California (Appendix 2).

Factors Predicting Antivenom Usage

The median number of vials of CroFab needed to resolve envenomations in our study was 12, which corresponds to a standard six vial initial dose and three maintenance doses of two vials each, according to the current dosing recommendation for CroFab (BTG International Inc. 2012; Lavonas et al. 2011). Our results are consistent with other research wherein the median number of vials needed for initial control was six (Lavonas et al. 2009).

The results of our analyses predicting antivenom use were similar to our analyses of overall severity and symptom progression, with snake size, patient mass, and iSSS all being significant predictors of antivenom use. These three variables were significant in four of our five ANCOVA models. None of these variables were significant in the model that tested for differences between snake species (model 2, Table 3), presumably because snake species explains some of the variation in snake size (see Chapter 2). Our analyses further suggest that length of the envenoming snake is the most important predictor of antivenom use, explaining ~18% of the variance (model 1), whereas patient mass and snake size had small to moderate effect sizes. In general, our statistical model (model 1; Table 5) showed that, on average (holding other factors constant), bites from large snakes required approximately 7 more vials of antivenom than those from small or medium snakes. Though effect sizes were small, the nature of the relationship between patient mass and iSSS was also described by our statistical models. Based on model 1 (Table 5), the coefficients of the underlying regression models suggested that, holding other factors constant, every 1 kg increase in patient mass was associated with a decrease in antivenom use by 0.04 vials. Likewise, every one point increase in iSSS was associated with an increase in antivenom use by 0.44 vials.

The two interactions (one in model 3 and one in model 4; Table 5) suggest some effect of the anatomical location of the bite and whether the bite was legitimate or not. The interaction plots (Figure 2) suggest that legitimate bites and bites to the lower limb by large snakes may require more antivenom. These two factors may be related, as more legitimate bites tend to happen to the lower extremities (Chapter 3). The ultimate reason for these relationships, however, remains unclear. Despite the moderate effect sizes of these interactions, the results may be statistical anomalies.

Three subscores of iSSS predicted antivenom dosage. Of these, the gastric subscore was the most predictive, though its effect size was only moderate. The statistical model suggested that, holding other factors constant, a one point increase in gastric subscore predicted a decrease in antivenom use by 2.77 vials. This finding differs from other research that found no relationship between gastrointestinal symptoms and bite severity (Thornton et al. 2012). It remains unclear why higher initial gastric subscores would predict a reduction in antivenom use, but gastrointestinal subscores are highest in bites from the Mohave rattlesnake (*C. scutulatus*), for which lower local wound scores could predispose physicians to prescribe less antivenom (Appendix 1). The neurological and hematological subscores had small effect sizes, with the statistical model showing that a one point increase in the neurological and hematological subscores resulted in a 1.33 and 1.06 vial increase in antivenom usage, respectively. We expected stronger relationships between initial local wound and initial hematological subscores and antivenom use, because these two symptoms, especially the former, are largely relied upon by physicians for antibody dosing.

Clinical Recommendations

Several conclusions can be drawn from our analyses that can be of use for clinicians in anticipating the overall severity of a bite, the degree of symptom progression, and the quantities of CroFab needed. In terms of overall severity and symptom progression, this study suggests that iSSS captures the vast majority of the variation in overall snakebite severity, and further suggests that, while most cases will show some symptom progression beyond initial assessment, such progression is unlikely to be extreme and will likely result from local wound symptoms. Further, we found that higher iSSS scores predict a reduced progression of symptoms, suggesting that clinicians be more vigilant in monitoring symptom progression in patients with lower severity scores.

Of greatest interest to clinicians may be our results related to the number of vials of CroFab needed to resolve symptoms, which may suggest changes to the way CroFab is currently administered. In our study, the factor with the greatest effect size was the length of the envenoming snake. We found that bites from large snakes (> 75 cm in length) required ~7 more vials of CroFab than bites from medium or small snakes. This roughly corresponds to the six-vial initial dose in the current dosing recommendations for CroFab (BTG International Inc. 2012; Lavonas et al. 2011). Therefore, clinicians may need to anticipate giving a second initial dose of six vials if it can be determined that the envenoming snake was greater than 75 cm in length.

Though the effect size was small, patient mass was also significantly related to the number of vials of CroFab needed, with smaller patients needing more vials of antivenom. This may suggest a need to re-examine the current treatment algorithm that advocates using the same dosing regimen for both pediatric and adult cases (BTG International Inc. 2012; Lavonas et al. 2011). A rule of thumb that might be suggested based on the regression coefficients in our statistical models would be that, for every 20 kg reduction in body mass, one extra vial of CroFab should be added to the first initial dose. Since the median patient mass for this study was about 75 kg, this would mean adding one extra vial for patients less than 55 kg, two vials for patients less than 35 kg, and three extra vials for pediatric patients less than 15 kg.

Initial snakebite severity (iSSS) was also significantly related to total vials of CroFab, though the effect size was rather small. In general, the regression coefficients from our statistical models suggested the need for an extra ~0.5 vials for every one point increase in iSSS. This may make the calculation of iSSS useful for clinicians seeking to determine whether a second initial dose is indicated. After the initial dose is administered, an iSSS of 8 or more may suggest that a second initial dose of four vials is warranted, whereas an iSSS of 12 or more may suggest a second initial dose of six vials may be indicated.

Limitations

One limitation of the conclusions of this study is its limited scope. We only collected data based on information recorded from the time of the envenomation until initial discharge from the hospital. Accordingly, our analyses do not inform prediction or treatment of potential further complications related to envenomation that may be manifested after initial discharge from the hospital. Potential complications following discharge include the recurrence of envenomation symptoms, which has been documented with the use of CroFab (Boyer et al. 1999; Boyer et al. 2001; O'Brien et al. 2009; Lavonas et al. 2014), and other long term physical and emotional morbidities (Dart et al. 1992; Smith & Bush 2010; Williams et al. 2011).

Despite our attempt to maintain the highest possible methodological rigor, several sources of bias are common to studies based on retrospective chart reviews. Bias may exist, for example, in the type and extent of missing data. More complete data may have been collected for patients with more severe bites, because these cases were likely to have received more attention from a greater number of clinicians, including one of our investigators (SPB). Also, charts did not specify whether recorded patient mass was measured or estimated. Since it may be more difficult to measure the mass of patients confined to a bed, a greater proportion of estimated masses may have been recorded for patients with more severe envenomations. Possible error existed in the information we relied on to determine size and species of the offending snake, and legitimacy of the bite. For information on the size and species of the snake, determinations based on photographs, specimens brought to the hospital, or measurement of fang-spread, especially those evaluated by SPB, were considered to be of high accuracy. Determinations from descriptions provided by other medical personnel (e.g. first responders), and the patients themselves, were likely to be less accurate. Cross-referencing the geographic location where the bite occurred with the known geographic ranges of southern California rattlesnakes provided some error mitigation for species determinations. Veracity of the patients was another potential source of error, particularly with respect to assigning bite legitimacy. Patients may have felt ashamed if their own lack of judgement resulted in the bite, or may have worried about legal ramifications, especially if they were keeping the snake in captivity, and, hence, may have given inaccurate information about how the bite occurred. In one case where a male patient was transferred to LLUMC from another medical facility, we found contradictory information surrounding the circumstances of the bite.

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CHAPTER FIVE

CONCLUSIONS

In this dissertation, I examined several aspects of human-rattlesnake conflict in southern California. I addressed the risks this conflict poses to both parties: the rattlesnakes and the humans. In this chapter, I will revisit the major findings in each study and suggest avenues for future research.

In Chapter 2, I investigated the impact of short-distance translocation (SDT) and long-distance translocation (LDT) on Red Diamond Rattlesnakes (*Crotalus ruber*) located near residential development in southern California. Depending on the metric measured (minimum convex polygon, local convex hull, range length), activity ranges of LDT snakes were 38.6–67.1% larger than those of SDT snakes, which, in turn, had activity ranges that were 77.0–152.9% larger than non-translocated (NT) snakes. Snakes moved closer to human modified areas during summer, and were translocated most often during that season at the behest of property owners. Both SDT and LDT snakes were more likely to move into human-modified areas subsequent to translocation than NT snakes. The distance a snake was translocated affected its risk of movement into human modified areas and its risk of returning to its site of capture, with every 1 m increase in distance resulting in a 1.2% decreased risk of moving into a human-modified area, and a 1.5% decreased risk of returning to the site of capture. We found no differences in the survival rate between translocated snakes (LDT and SDT) and NT snakes.

Based on these findings, I suggest that translocation may be a viable approach to reduce human-snake conflict. However, the success of this approach may depend on the local ecology and the biology of the rattlesnake species. I proposed that snakes which rely on specific (typically communal) hibernacula will be placed at greatest peril with translocation, as these snakes may experience difficulty locating a suitable site for overwintering. Snakes that do not rely on specific hibernacula for brumation (≈ hibernation), such as those included in our study, may be less effected by translocation.

In Chapter 3, I investigated the effects of a number of factors on the etiology and severity of envenomations among victims of rattlesnake envenomations in southern California. I conducted a retrospective review of 354 cases of venomous snakebite admitted to Loma Linda University Medical Center (LLUMC) between 1990 and 2010. In terms of etiology, I found that 80.5% of snakebite cases were male victims, 69.2% of bites were to an upper limb, and 88.0% were distal to the wrist or ankle. Of 308 cases where a determination could be made, 45.8% were illegitimate (i.e., bites provoked by the human interacting with the snake). Most snakebites occurred during the spring mating season, followed by another large pulse during fall associated with newborn snake emergence. Males snakebite victims were 2.9, 7.1, and 3.1 times more likely to sustain bites to the upper extremity, distal to the ankle or wrist, and via illegitimate provocation, respectively, than female victims. Those admitting to alcohol or drug use were 5.7 times more likely to sustain illegitimate bites, which were 111.0- and 7.1-fold more likely to be to the upper limb and distal to the ankle or wrist, respectively. Snakebite severity was positively associated with snake size, negatively associated with patient mass, and independent of patient age, snake taxon, anatomical location of bite, legitimate versus illegitimate (provoked) bites, and time until hospital admission. The effectiveness of CroFab antivenom against each of the seven southern California rattlesnake taxa was also assessed. Despite concerns that CroFab is ineffective in neutralizing the venom of some snake taxa, especially that of the Southern Pacific Rattlesnake (*Crotalus oreganus helleri*; Consroe et al., 1995; Sánchez et al., 2003), we found its clinical effectiveness to be similar for all taxa.

In Chapter 4, I further investigated rattlesnake envenomations in southern California from the perspective of a clinician, assessing several factors currently omitted from snakebite treatment algorithms (Lavonas et al. 2011) for their potential usefulness as predictors of overall snakebite severity score, symptom progression from initial assessment, and antivenom use. The factors were the same as those for the previous chapter. I found initial snakebite severity score (iSSS), the size of the envenoming snake, and patient mass to be significant predictors of overall bite severity, symptom progression, and antivenom use. Initial SSS proved to be the most effective predictor of overall severity, explaining ~70% of the variance. Snake size best predicted symptom progression, with larger snakes inflicting greater symptom progression. Patient mass and iSSS also significantly predicted symptom progression, with smaller patients and those with lower iSSSs experiencing greater symptom progression. Snake size was also the most important factor predicting antivenom use, with large snakes requiring, on average, seven more vials of CroFab than medium or small snakes. I further evaluated whether scores of each of six symptom classes assessed upon admission (iSSS subscores for cardiovascular, gastric, hematological, local wound, neurological, and pulmonary symptoms) influenced overall SSS, symptom progression, and antivenom use. All six subscores significantly predicted overall severity, but hematological, neurological, and cardiovascular subscores were most salient. Local wound score was the only significant predictor of symptom progression. Gastric, neurological, and hematological symptoms were significantly associated with antivenom use, but the gastric subscore showed an unexpected inverse relationship to antivenom use. Based on our analyses, we suggest that iSSS, snake size, and patient mass are especially useful to clinicians for anticipating antivenom needs. I also suggested several potential rules of thumb that could be added to the current snakebite treatment algorithm to help clinicians anticipate antivenom needs.

Future Directions

In Chapter 2, I examined the effect of long- and short-distance translocation in Red Diamond Rattlesnakes in southern California. While this study represents one of the most complete studies to date on translocation in a rattlesnake species, substantially more research is needed to improve our understanding of the impacts of mitigation translocation on snakes. In spite of accumulating studies on the effects of translocation on rattlesnakes (see Table 1, Chapter 2), this form of mitigation remains a highly experimental approach for which generalizations should be made with caution. Studies vary substantially in their translocation protocols, duration, and assessments of behavior and mortality, and all are constrained by relatively small samples, including mine.

Comparing the low mortality in my study with the higher mortality seen in other studies (e.g. Reinert & Rupert, 1999; Nowak, Hare, & McNally, 2002) suggests that environmental and ecological conditions play an important role in mortality due to translocation, and failure to account for this may influence our assessment of the viability of mitigation translocation in rattlesnakes. Clearly, more studies of this kind are needed from a wide variety of species and habitats.

My results from the snakebite studies of Chapters 4 and 5 also suggest avenues for future research. The conclusions drawn from these studies were made on the basis of a retrospective review of medical records. The conclusions we derived have yet to be tested in a prospective study. Such studies are needed to test the validity of my conclusions and to determine the utility of the potential rules of thumb I suggest to help clinicians anticipate antivenom needs. The approaches I used to assess snakebite severity and its amelioration can be adapted to future studies in North America and other regions, and studies of new antivenoms and novel treatments as they emerge.

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APPENDIX A

FROM VENOME TO SYNDROME: CORRESPONDENCE OF RATTLESNAKE VENOM COMPOSITION AND CLINICAL SYMPTOMS OF SNAKEBITE

Aaron G. Corbit and William K. Hayes

Abstract

Rattlesnakes possess highly variable venoms that cause severe systemic and local tissue effects in human snakebite victims. We sought to determine the degree to which different clinical symptoms could be attributed to variation in the venom composition of seven southern California rattlesnake taxa. To compare species differences in clinical symptoms resulting from bites, we assigned snakebite severity subscores (SSsS) to 204 envenomated patients presenting at the LLU Medical Center. This sample included only cases with positive identification of the offending snake species. We quantified SSsS using a standard scale that included effects on local tissue, cardiovascular, respiratory, gastrointestinal, hematological, and neurological systems. Discriminant function analysis using equal probabilities for group assignments and controlling for snake size yielded a highly significant model ($\Lambda = 0.39$, $\chi^2_{42} = 148.63$, $P < 0.001$; $N = 166$ cases with complete data). Overall, 50.0% of cases were correctly classified to snake species, which greatly exceeded that expected from random (14.3%). To characterize venom variation, we subjected at least one sample from each rattlesnake taxon to high-pressure liquid chromatography (HPLC) fractionation, which allowed us to identify the relative

composition of major toxin families. Major species differences in SSsS corresponded to obvious differences in the venom composition. As examples, (1) the high neurological subscores of *Crotalus oreganus helleri* bites corresponded to high levels of myotoxins in their venom that caused frequent muscle fasciculations and/or myokymia; (2) the high pulmonary, cardiovascular, and gastrointestinal subscores of *C. scutulatus* corresponded to high levels of Mojave toxin, a presynaptic neurotoxin; (3) the high local wound scores of *C. mitchellii* and *C. o. oreganus* bites probably corresponded to high levels of snake venom serine proteases (SVSPs) and cysteine-rich secretory proteins (CRiSPs); and (3) the high hematological subscores of *C. atrox* and *C. ruber* bites corresponded to high levels of metalloproteases. We further documented flaccid paralysis and fasciculations/myokymia—symptoms expected of bites from *C. scutulatus* and *C. o. helleri* that are known to possess neurotoxins in their venoms—in occasional bites from several taxa that presumably lack neurotoxins. Collectively, these findings offer valuable insights on how venom composition influences clinical symptoms, and can inform the design of more effective antivenoms and treatment algorithms for rattlesnake bites.

Introduction

Rattlesnakes possess highly variable venoms that can cause a variety of severe local tissue, hematological, and neurological effects (White et al. 2003; Boyer et al. 2015). Seven rattlesnake taxa occur in southern California (see Chapter 3), including two, the Southern Pacific Rattlesnake (*Crotalus oreganus helleri*) and the Mohave Rattlesnake (*Crotalus scutulatus*), that are known to cause neurotoxic symptoms. However the neurological effects of these two taxa differ. *Crotalus o. helleri* envenomations cause muscle fasciculations and/or myokymia (Wingert & Chan 1988; Bush & Siedenburg

1999), whereas those of *C. scutulatus* often cause flaccid paralysis due to the action of a well characterized dimeric, presynaptic, phospholipase A_2 neurotoxin known as Mojave toxin (Farstad et al. 1997; Massey et al. 2012). A population of *C. o. helleri* around Mount San Jacinto possesses a homologous phospholipase A_2 neurotoxin (French et al. 2004; Sunagar et al. 2014), but other than a case report for a dog bitten in the San Jacinto Mountains (Hoggan et al. 2011) and a case of possible flaccid paralysis in a Caracal (*Caracal caracal*; Singleton et al. 2009), no flaccid paralysis resulting from *C. o. helleri* envenomation has been documented.

Whereas a growing body of research characterizes the differences in venom composition among rattlesnake taxa (Calvete et al. 2010; Mackessy 2010; Massey et al. 2012; Sunagar et al. 2014), much less work has been done to document differences in the clinical syndrome that can be attributed to variation in venom composition. We can expect that venom components present in the largest quantities of a given venom will elicit the most severe clinical symptoms. Thus, if a snake has a large component of neurotoxin in its venom, then we would expect the clinical presentation to feature substantial symptoms related to neurotoxicity. Prior studies from southern California suggest that the overall snakebite severity score (SSS) does not differ among rattlesnake taxa (Janes et al. 2010; Chapter 3 of this dissertation). However, some differences in specific clinical symptoms (snakebite severity subscores) have been documented among a few of these same taxa (Janes et al. 2010), and even among venom phenotypes of a single species (Massey et al. 2012).

The purposes of this study were to (1) examine the extent to which clinical symptoms differ among seven rattlesnake taxa in southern California, and (2) to identify candidate toxin families largely responsible for these differences. This preliminary report represents the largest and most comprehensive study to date in attributing the clinical syndrome of rattlesnake envenomation (i.e., the specific set of clinical symptoms) to venom composition. We are currently expanding the study to include detailed proteomic analyses of a much larger set of venom samples.

Materials and Methods

Snakebite Severity Subscores

We utilized a subset of 204 cases from the retrospective dataset of 354 cases used in Chapters 3 and 4 wherein the taxon of the envenoming snake was known. Seven rattlesnake taxa were represented: Western Diamondback (*C. atrox*), Sidewinder (*C. cerastes*), Southwestern Speckled Rattlesnake (*C. mitchellii pyrrhus*), Northern Pacific Rattlesnake (*C. o. oreganus*), Southern Pacific Rattlesnake (*C. o. helleri*), Red Diamond Rattlesnake (*C. ruber*), and Mojave Rattlesnake (*C. scutulatus*). Besides snake taxa, we included information about the size of the snake that bit each patient, as well as the snakebite severity scores following the rubric designed by Dart et al. (1996) for adults and an adjusted rubric for pediatric patients (see Chapter 3). This scoring method, which ranges from 0–20 points (higher scores indicating more severe bites), is based on the objective evaluation of clinical parameters in six categories: local wound effects, hematologic (coagulation) parameters, and symptoms associated with the pulmonary, cardiovascular, gastrointestinal, and central nervous systems. Snakebite severity subscores (SSsS) for each of these categories, which range from 0–3 or 0–4, were recorded in this dataset as well as a summed total score. Two sets of these scores were recorded in the dataset. Initial scores were calculated by determining the maximum scores for each symptom category based on information recorded from time of the bite until the patient received their first dose of antivenom. Maximal scores were determined by taking the maximum scores for each symptom category based on information recorded from the time of the bite until initial discharge from the hospital. However, we only report analyses based on maximal SSsS here.

The dataset also characterized the presence or absence of flaccid paralysis and fasciculations/myokymia. The presence of flaccid paralysis was assigned if case documentation noted muscle weakness, ptosis, slurred speech, or other signs of motor impairment due to loss of muscle tone. The presence of fasciculations/myokymia was assigned if any mention of uncontrolled muscle twitching was made in the medical record. Symptoms were assumed to be absent if undocumented. Some researchers distinguish between fasciculations and myokymia (Gutmann & Gutmann 2004) both of which manifest as spontaneous, fine, involuntary undulating waves or ripples of muscle fibers that are often visible beneath the skin; however, it may be difficult to distinguish these clinically (LoVecchio et al. 2005), and the distinction remains unclear in the snakebite literature.

Venom Composition

We created a representative chromatogram for each snake taxon by fractionating a venom sample using reversed-phase high-pressure liquid chromatography (RP-HPLC). Methods are described elsewhere (Sunagar et al. 2014; Gren 2015). This preliminary work involved a single venom sample for most of the taxa, but we are currently running additional venom samples to obtain a better understanding of geographic variation within each taxon. All venom samples analyzed here were obtained within the same geographic region where snakebites occurred. Venom composition can be inferred from the chromatograms, with several major toxin families appearing in distinct portions of the chromatogram. Most notably, small basic proteins comprising myotoxins (β-defensins) appear with the early eluents (roughly 56-65 mL), Mojave toxin appears as two peak sets (for the two subunits) within the 78–95 mL region, serine and numerous other proteases and toxins emerge within the 95–123 mL region, and snake venom metalloproteinases dominate beyond 123 mL (Fig. 1).

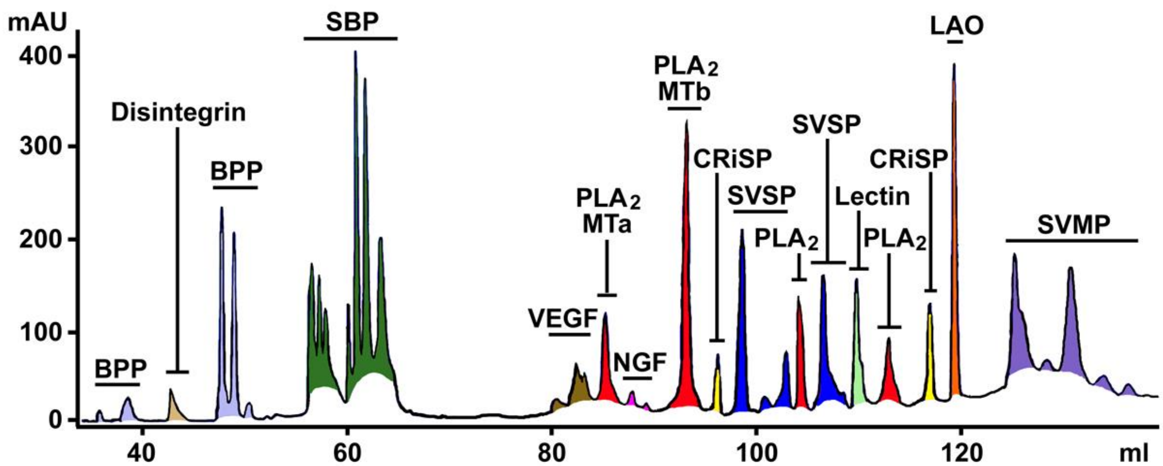

Figure 1. Composite RP-HPLC chromatogram, combining peaks from several *Crotalus oreganus helleri* venom samples to illustrate major toxin protein families. Presence and order of elution of proteins can vary substantially among some toxin families, particularly within the range of 95–120 mL. BPP = bradykinin potentiating peptide; SBP = small basic peptides comprising myotoxins; VEGF = vascular endothelial growth factor; PLA_2 = phospholipases A_2, including Mojave toxin (MT), a dimeric presynaptic neurotoxin with acidic (MTa) and basic (MTb) subunits; CRiSP = cysteine-rich secretory protein; SVSP = snake venom serine protease; LAO = L-amino acid oxidase; SVMP = snake venom metalloproteinase. Figure modified from Gren (2015). The three entities most reliably identified by position are SBP, MT, and SVMP.

Statistical Analyses

We performed discriminant function analysis (DFA) on the SSsS for each case to examine whether there were differences in the clinical symptomology among snake species. Prior to analysis, we screened the data to assure they largely met parametric assumptions. We based our analyses and conclusions on a DFA model that assumed equal prior probabilities for each group. DFA models that use equal prior probabilities are known to be less biased (Mertler & Vannatta 2004); however, we also conducted DFAs using prior probabilities computed from group sample sizes for comparison and obtained similar results. We included size of the snake in the model to increase the predictive success of the model and to control for species differences in snake size. Cases wherein the size of the snake could not be determined were omitted, leaving a final sample of 166 cases for this analysis. Similar multinomial logistic regression models using each species as a reference were also performed. These models had somewhat better prediction success; however, the DFA yielded similar results and provided a canonical plot of discriminant scores to visualize species differences. Only the DFA results are reported here. We standardized the SSsS values for graphical presentation to illustrate species differences relative to all snakebites. These standardized values do not control for snake size.

We further tested for differences in the incidence of flaccid paralysis and fasciculations/myokymia among species using Fisher exact tests due to small sample sizes for some species. We conducted the DFA using SPSS 13.0 (SPSS Inc., Chicago, IL), and Fisher exact tests via R 3.2.1 (R Core Team 2014). Alpha was set to 0.05. We present results as means ± 1 S.E.

Results

Standardized means for each SSsS for each species are shown in Figure 2. For some taxa, one or several clinical symptoms averaged much higher subscores relative to the average snakebite. Again, these values are not adjusted for snake size.

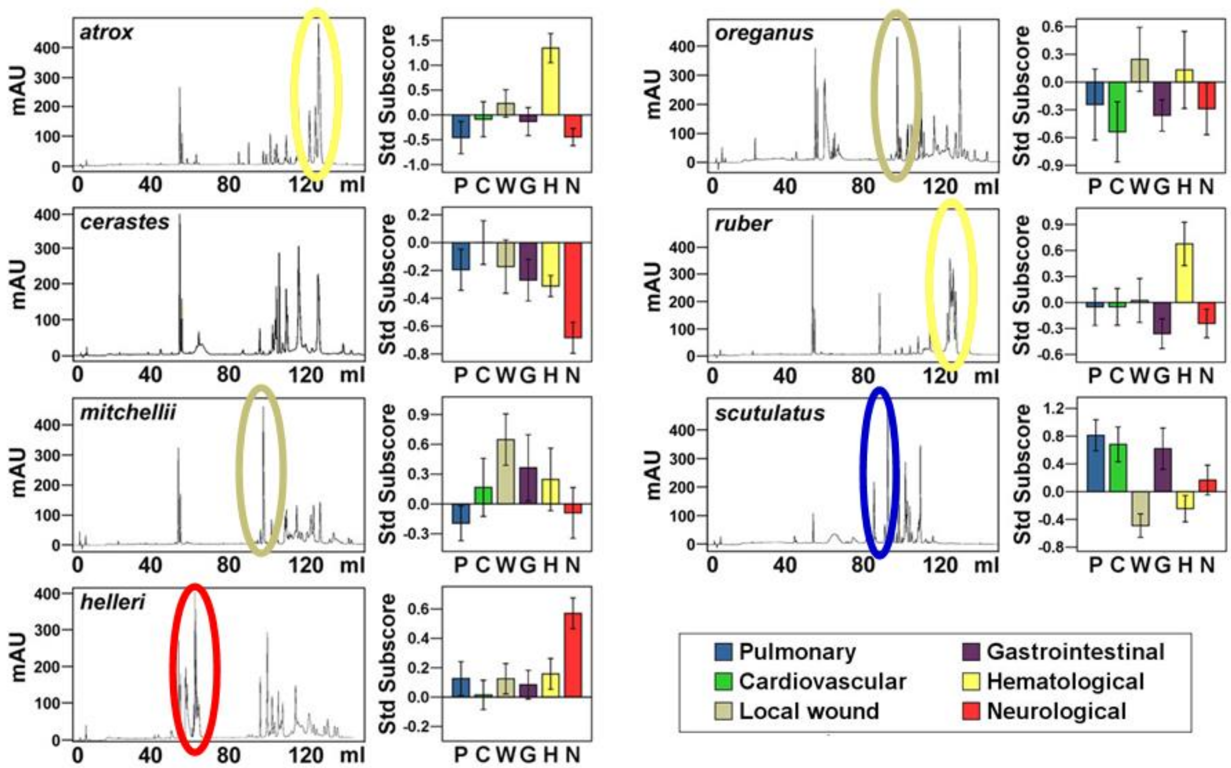

Figure 2. RP-HPLC chromatograms of venom samples paired with standardized SSsS (mean ±1 SE) of clinical symptoms from each of seven rattlesnake taxa. Colored ellipses indicate correspondence between elution peaks and clinical symptoms. For SSsS, n = 7 to 74 for each taxon.

Wilks' lambda for the DFA model was significant ($\Lambda = 0.390$, $\chi^2_{42} = 148.633$, $n = 166$, $P < 0.001$), indicating that SSsSs differed among the snake taxa. Separation of snake species based on the first two functions is depicted in Figure 3. The first function (43.2%

of variance) was positively associated with the hematological (standardized coefficient = 0.910) and local wound (0.303) components. It was negatively associated with the pulmonary (-0.454), cardiac (-0.382), and gastric (-0.279) components. This function did well separating *C. atrox* and *C. ruber,* which tended to cause significant hematologic and local wound symptoms, from *C. scutulatus*, which tended to cause more pulmonary and cardiac symptoms. The second function (34.2% of variance) was strongly and positively associated with the neurological component (1.090) and negatively associated with the cardiac (-0.279) and pulmonary (-0.264) components. The second function separated out *C. o. helleri*, which tended to cause more significant neurological symptoms, and *C. cerastes*, which had the lowest subscores.

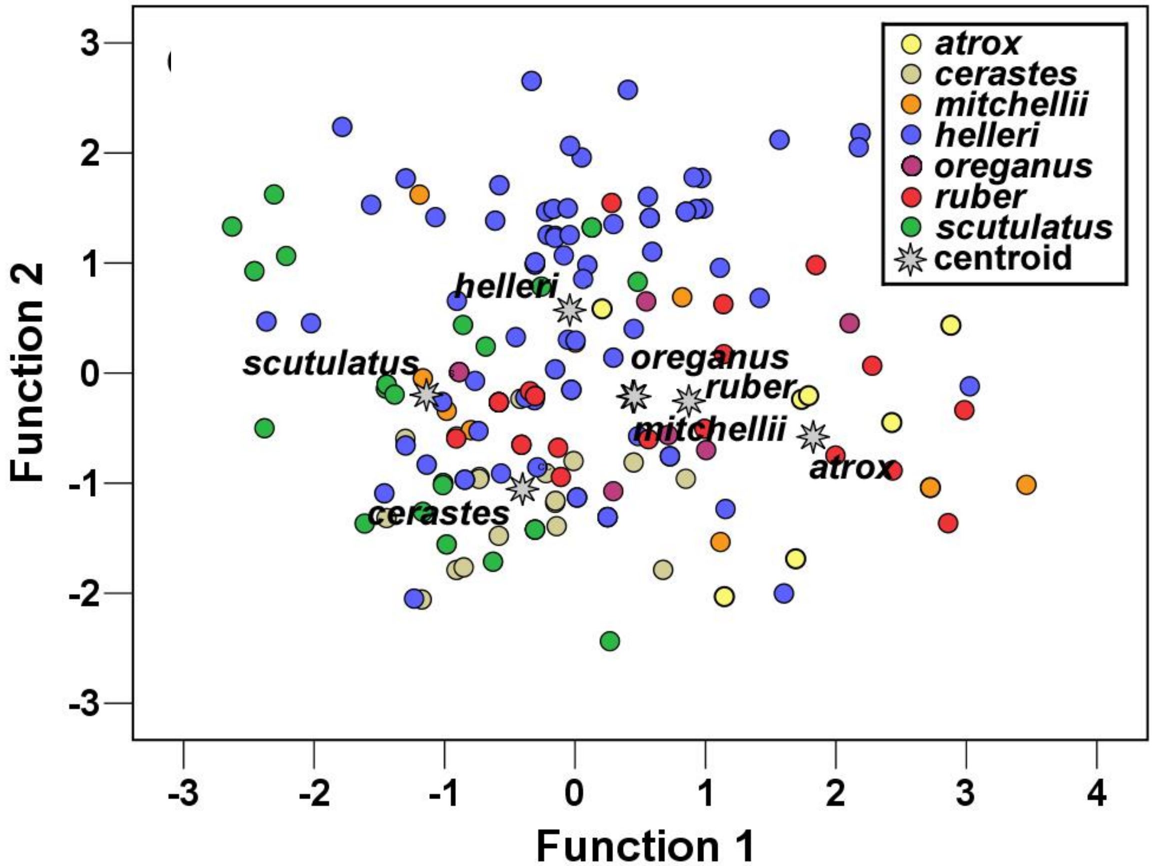

Figure 3. Canonical plot of discriminant scores for snakebite severity subscores (SSsSs) from each of seven rattlesnake (genus *Crotalus*) taxa. Function 1 (48.6% of variance) and Function 2 (29.6% of variance) were comprised primarily of hematological and neurological subscores, respectively.

Classification results for the DFA model indicated that 50.0% of the snakebite cases were classified correctly to snake species, and somewhat fewer (44.6%) were cross-validated using leave-one-out. Accuracy for each species was *C. atrox* 87.5%, *C. cerastes* 80.8%, *C. mitchellii pyrrhus* 33.3%, *C. o. helleri* 44.2%, *C. o. oreganus* 14.3%, *C. ruber* 33.3%, and *C. scutulatus* 52.4%. Classifications greatly exceeded those expected from random, which was 14.3% based on the assumption that prior probabilities were equal for all species. The only exception was for *C. o. oreganus*, for which model accuracy was equal to what was expected by chance. When the DFA was run using prior probabilities computed from group sizes, classification success improved to 60.0% and 52.4% for original and cross-validated cases, respectively.

Major species differences in SSsSs corresponded to obvious differences in venom composition (Fig. 2). As examples, (1) the high neurological subscores of *C. o. helleri* bites corresponded to high levels of myotoxins in the venom; (2) the high pulmonary, cardiovascular, and gastrointestinal subscores of *C. scutulatus* corresponded to high levels of Mojave toxin in the venom; (3) the high local wound scores of *C. mitchellii* and *C. o. oreganus* bites probably corresponded to high levels of snake venom serine proteases (SVSPs) and cysteine-rich secretory proteins (CRiSPs), and (4) the high hematological subscores of *C. atrox* and *C. ruber* bites corresponded to high levels of metalloproteases in the venom.

The number of cases showing symptoms consistent with venom-induced flaccid paralysis and muscle fasciculations/myokymia for each rattlesnake species is shown in Table 1. Fisher exact tests showed significant differences among species for both flaccid paralysis ($P = 0.022$) and fasciculations/myokymia ($P < 0.001$). Envenomations from only two taxa showed symptoms indicative of flaccid paralysis. These were *C. scutulatus* and *C. o. helleri*, with 21.4% and 3.2% of cases showing these symptoms, respectively. Fasciculations/myokymia were documented in four taxa, with *C. o. helleri* having the highest proportion of cases exhibiting these symptoms (45.3%). Other taxa showing fasciculations/myokymia were *C. scutulatus* (14.3%), *C. ruber* (9.5%), and *C. mitchellii pyrrhus* (8.3%).

Table 1. Envenomation cases showing either flaccid paralysis or fasciculations/myokymia by envenoming rattlesnake species in southern California

Rattlesnake Species	Total Cases	Flaccid Paralysis (%)	Fasciculations (%)
Mohave Rattlesnake	28	6 (21.4)	4 (14.3)
Northern Pacific Rattlesnake	7	0 (0)	0 (0)
Red Diamond Rattlesnake	21	0 (0)	2 (9.5)
Sidewinder Rattlesnake	32	0 (0)	0 (0)
Southern Pacific Rattlesnake	95	3 (3.2)	43 (45.3)
Speckled Rattlesnake	12	0 (0)	1 (8.3)
Western Diamondback Rattlesnake	9	0 (0)	0 (0)

Note: We cannot verify that all cases showing these symptoms were caused by the effects of the venom

Discussion

Rattlesnake venoms contain a variety of protein-based toxins, and these can vary substantially among and within different taxa. Chromatograms from this preliminary analysis represent the first proteomic assessment for a number of species examined (*C. cerastes*, *C. o. oreganus*, *C. mitchellii*, *C. ruber*), and confirm the substantial differences in venom composition among southern California rattlesnake taxa. Although geographic venom composition has been documented in several of the remaining taxa (*C. scutulatus*: Massey et al., 2012; *C. o. helleri*: Gren, 2015), our ongoing analyses of additional venom samples from each taxon suggest that the chromatograms portrayed in Fig. 2 are largely representative for southern California specimens. Whereas previous studies showed that the overall severity of rattlesnake envenomation does not differ among southern California rattlesnake species (Janes et al. 2010; Chapter 3), this study revealed significant differences in envenomation symptomology among these species, which we can attribute, in part, to venom composition differences.

Correspondence of Venom Composition and Clinical Symptoms

Perhaps the most surprising finding of the study was the high level of symptoms heretofore attributed largely to neurotoxicity (Ranawaka et al. 2013) that accompanied bites from *C. o. helleri*. Indeed, the neurological subscores of *C.o. helleri* exceeded those of *C. scutulatus*. This difference was unexpected because the only documented presynaptic neurotoxin within the genus, Mojave toxin and its homologues (Werman 2008), is believed to be present throughout the range of *C. scutulatus* in California, but only in one small portion of the range of *C. o. helleri* (the San Jacinto Mountains; French et al. 2004; Gren 2015). Most of the bites from *C. o. helleri* in this study were from regions lacking this neurotoxin. The high neurological subscores resulted because of the high frequency of fasciculations/myokymia, which are scored as neurotoxicity in the original SSS rubric (Dart et al. 1996). Fasciculations/myokymia occurred in 45.3% of all bites by this taxon, and were associated with the relatively high proportion of small basic myotoxins in the venom. Heretofore, the toxins that cause fasciculations/myokymia in rattlesnake bites have not been clearly identified (Vohra et al. 2008), though Ranawaka et al. (2013) suggested the involvement of crotamine, which occurs among the small basic myotoxins present in *C. o. helleri* venom (Sunagar et al. 2014; Gren 2015; see also Salazar et al. 2009). Our study strongly implicates a role for myotoxins in causing these symptoms. Although some of these myotoxins may also be neurotoxins (Gren 2015), the myotoxins may be causing fasciculations/myokymia independent of neurotoxicity.

The low level of overtly neurotoxic symptoms documented for *C. scutulatus* was also surprising (only 21.4% of cases). Mojave toxin is absent from the venom of *C. scutulatus* from south-central Arizona (Massey et al. 2012), but occurs in the venom elsewhere within the species' range, including southern California (Ho & Lee 1981; Glenn & Straight 1989). Accordingly, previous literature characterizes the envenomation symptomology associated with *C. scutulatus* as largely neurotoxic symptoms such as paresthesia of the face and limbs, respiratory arrest, lethargy, diplopia, ataxia, seizures, and altered consciousness though edema, ecchymosis, and pain, with rhabdomyolysis, myoglobinuria, and renal failure also documented (Jansen et al. 1992; Farstad et al. 1997). However, our analyses portray a more distinct envenomation syndrome for specimens possessing Mojave toxin that is largely lacking from the literature, with unremarkable neurotoxic symptoms as scored by SSS, relatively severe pulmonary, cardiovascular, and gastrointestinal symptoms, and relatively mild local wound and hematological symptoms compared to other rattlesnake taxa. The low levels of neurotoxic symptoms documented in our study may simply be the result of clinicians failing to detect or document the neurotoxic effects of Mojave toxin. While clinicians are likely to detect extreme neurotoxic symptoms associated with severe envenomations from this species, the effects of this toxin in moderate or mild envenomations may be less obvious, especially in patients lying relatively motionless in hospital beds. In prior studies, Farstad et al. (1997) detected neurotoxic symptoms in 69.2% of 13 presumed *C. scutulatus* bites in California, whereas Massey et al. (2012) reported neurotoxicity in only 9.3% of 75 bites from *C. scutulatus* in a region of Arizona where they possess Mojave toxin. The fact we detected more severe pulmonary and cardiac symptoms in *C.*

scutulatus envenomations is consistent with the neurotoxic effects of Mojave toxin, as partial paralysis of the diaphragm would be expected to cause shallow, rapid respirations and tachycardia.

Minimal and even delayed local tissue injury from *C. scutulatus* specimens having Mojave toxin have been noted previously (Russell 1969; Glenn et al. 1983; Wingert & Chan 1988), which could lead to under-treatment with antivenom (Jansen et al. 1992). Current treatment recommendations and algorithms for rattlesnake bite require monitoring of local wound effects—notably swelling—and blood abnormalities for dosing decisions regarding antivenom (Lavonas et al. 2011). Our results confirm the need for practitioners to recognize the very distinct envenomation syndrome of bites resulting from venoms having Mojave toxin or its homologues, as failure to recognize the symptoms of severe envenomation could lead to under-treatment.

The relatively high hematological subscores of *C. atrox* and *C. ruber* can be attributed to the high metalloproteinase content of their venoms (Fig. 2; see also Calvete et al. 2009). Snake venom metalloproteinases (SVMPs) have well-documented hemorrhagic activities that disrupt cardiovascular function and impair hemostasis (Takeda et al., 2012; Markland and Swensen, 2013; Casewell et al., 2015). These two taxa appear to have the highest proportions of SVMPs present in the venoms of the seven snake taxa we examined. The strong correspondence between SVMP presence and bleeding abnormalities suggests a major role of SVMPs in hemostasis disruption of human snakebite victims.

Our results largely fit the trend seen in rattlesnakes (genus *Sistrurus* and *Crotalus*) wherein venoms are either highly coagulopathic and tissue destructive, and have lower overall toxicity (type I venoms), or are highly toxic (often due to the presence of Mojave toxin or its analogs) and have less coagulopathic and tissue destructive properties (type II venoms; Mackessy 2008; Mackessy 2010). Consistent with Mackessy (2008), our analysis suggests that *C. atrox*, *C. mitchellii*, *C. o. oreganus*, and *C. ruber* have envenomation symptomologies that would be expected for a type I venom. Envenomations from *C. cerastes* showed substantially reduced standardized subscores compared southern California taxa. This probably resulted more from snake size than anything else, as the species averages smaller in body size than all other southern California rattlesnakes (Klauber 1972; Campbell & Lamar 2004). The lack of neurotoxic symptoms documented for this species would suggest that it has venom more consistent with the type I profile, which has been confirmed by Mackessy (2008).

Our study revealed several cases where the envenoming taxon was associated with uncharacteristic neurotoxic symptoms. These include three cases of bites attributed to *C. o helleri* causing flaccid paralysis in areas distant from San Jacinto area, the only known region where snakes of this subspecies contain the Mojave toxin analog. We also documented fasciculations/myokymia in four cases of bites attributed to *C. scutulatus*, two cases attributed to *C. ruber*, and one attributed to *C. mitchellii*. Russell (1960) reported occurrences in some bites from *C. atrox* in southern California. While these cases are interesting, they must be interpreted with caution. First, while many snake identifications in this study were made by reputable individuals (see Chapter 3), there is the possibility that some of the envenoming snakes were misidentified. Second, given the nature of this study, we cannot say with certainty that these symptoms were caused by the effects of the venom. Other factors, such as alcohol use, extreme anxiety, and medications administered during the course of hospital treatment, may have contributed to or perhaps even elicited these symptoms. However, since significant venom variation is already known for two southern California taxa (Massey et al. 2012; Gren 2015), these unusual cases could be result of such variation. The findings we report here argue for further study of intraspecific venom variation within rattlesnakes, as such variation can have significant clinical effects.

Conclusions

This study, which compared the clinical symptoms and venom composition of seven rattlesnake taxa, provides the largest and most comprehensive study to date attributing the clinical symptoms of snakebite to venom composition. Our results suggest that clinicians may be underestimating the severity of mild or moderate envenomations from rattlesnakes with neurotoxic (Type II) venom, as they may fail to detect the direct neurotoxic effects of the venom and may inappropriately base their treatment decisions on hematological and local wound symptoms, resulting in under-treatment. Overall, this study shows distinct differences in envenomation symptomology between southern California rattlesnake taxa which can be correlated with venom composition differences between species. These findings can inform the design of more effective treatment algorithms and antivenoms for snakebite.

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APPENDIX B

CONSTIPATION ASSOCIATED WITH BRUMATION? INTESTINAL OBSTRUCTION CAUSED BY A FECALITH IN A WILD RED DIAMOND RATTLESNAKE

(*CROTALUS RUBER*)

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Summary

This report describes the fecalith-induced intestinal obstruction of a free-ranging red diamond rattlesnake (*Crotalus ruber*) and the snake's subsequent history following surgical removal of the fecalith. The captured snake exhibited an abnormally distended abdomen and an extremely hard mass, detected via palpation, near its vent. Coeliotomy yielded a 2.5-cm, 5-g fecalith from the large intestine. Microscopic dissection of the fecalith revealed no evidence of gastrointestinal parasitic worms. Subsequently, we implanted a radio-transmitter that allowed us to track the snake's movements for 7 months (until the radio signal vanished), indicating normal behavior, complete recovery, and good health apart from the obstruction. This observation suggests that fecalith development and intestinal obstruction represent potential risks of long-term fecal retention, an unusual physiological trait well documented among rattlesnakes and other stout, heavy-bodied terrestrial viperid snakes. Dehydration and decreased gut motility associated with brumation (≈hibernation) may predispose temperate snakes to fecalith formation. Regional drought and a small mammal diet with indigestible hairs might have also promoted fecalith formation in this specimen.

Key words

Brumation; Crotalinae; Fecal retention; Gastrointestinal; Serpentes; Viperidae

Introduction

Gastrointestinal obstruction and subsequent abdominal distension in snakes may be caused by several pathological conditions, including parasitism, blockage from a tumor, abscess, granuloma, or foreign body, or fecal impaction (Diaz-Figueroa and Mitchell, 2006). Snakes presenting with such obstructions may show significant lethargy, emaciation, and dehydration (Souza et al., 2004; Diaz-Figueroa and Mitchell, 2006). Gastrointestinal obstructions have been documented in both captive and wild snakes, with most wild examples involving black ratsnakes (*Pantherophis* spp., formerly *Elaphe obsoleta*) invading chicken coups and ingesting objects they mistake as eggs (e.g. Smith, 1953; Adams and Sleeman, 2005). Conventional wisdom dictates surgical removal of the obstructing object from the gastrointestinal tract to preserve the snake's life.

In this paper, we describe the first reported case of such an obstruction, caused by an impacted fecalith, in a large-bodied terrestrial pitviper (Serpentes: Viperidae: Crotalinae): a red diamond rattlesnake (*Crotalus ruber*). This species, attaining a length of up 1200 mm snout-vent length, occupies Mediterranean and xeric habitats from southern California, USA, south to the tip of the Baja Peninsula of Mexico, including several Pacific and Gulf of California islands (Beaman and Dugan, 2006). As a relatively sedentary species that frequently employs ambush tactics, all age groups feed largely on rodents and small mammals, with occasional lizards and birds also consumed (Brown et al., 2008; Dugan et al., 2008; Dugan and Hayes, 2012). The intestinal obstruction in this case report is particularly unusual in that it involved a wild-caught snake showing no evidence of gastroinstestinal parasite infection.

Case Report

As part of an ongoing radio-telemetry study of red diamond rattlesnakes in Loma Linda, California, USA, we collected an adult female rattlesnake (102 cm snout-vent length, 750 g) on 13 April 2010 at 1053 hr. A telemetered male snake was courting the female by chin-rubbing on the female's dorsum (Hayes, 1986) prior to her capture. Both specimens were observed exposed on a grass-covered, northwest-facing slope near a clump of California buckwheat (*Eriogonum fasciculatum*).

We brought the female into the laboratory for examination and transmitter implantation. Upon initial examination, palpation of the animal revealed an extremely hard mass about 10 cm anterior to the vent. The area anterior to the hard mass appeared swollen and abnormally distended, suggesting a possible bowel obstruction. Concerned about the snake's health, we kept the specimen in the laboratory at 23°C in a 50.8 × 27.9 × 33.0 cm (L × D × H) glass terrarium with newspaper substrate, ambient (low) humidity (due to a screen lid), an electrical heating pad adhered to a portion of the bottom, and no food. The snake's failure to defecate after 1 week prompted us to surgically remove the potentially obstructing object on 21 April.

The surgical procedure largely followed previously published surgical transmitter implantation methods (Reinert and Cundall, 1982; Hardy and Greene, 1999; Hardy and Greene, 2000). We anesthetized the snake by restraining it within a clear plastic tube (Midwest Tongs, Independence, MO, USA) and injecting 3 mL sevoflurane (SevoFlo, Abbott Laboratories, North Chicago, IL, USA) into a gauze plug at the distally sealed end of the tube. Once the snake reached the surgical plane of anesthesia, we placed it in a right lateral recumbent position and made a ca. 4-cm longitudinal incision between scales

2 and 3 on the left side of the snake and over the obstructing object. We gained access to the peritoneal space and intestine via an incision through the ventral abdominal muscles immediately ventral to the costal cartilage. The intestine appeared normal with no signs of inflammation or necrosis. A 1-cm incision in the large intestine allowed us to remove a solid fecalith measuring 2.5 × 2.0 × 2.0 cm and weighing 5 g . We closed the intestine with synthetic absorbable suture (Vicryl, Ethicon, Somerville, NJ, USA) in a simple continuous pattern. We then lavaged the peritoneal space with chlorhexadine solution (Phoenix Pharmaceutical Inc., St. Joseph, MO, USA) and packed it with nitrofurazone ointment (Fura-Zone, Squire Laboratories Inc., Revere, MA, USA) prior to closing the skin with Vicryl absorbable suture using an interrupted horizontal mattress pattern. We returned the snake to its terrarium with the electrical heating pad, and noted an uneventful recovery.

We dissected the fecalith and thoroughly examined it microscopically for evidence of an exceptional load of parasites, which has been documented previously as a cause for impaction in reptiles (Kane et al., 1976; de la Navarre, 2002; Diaz-Figueroa and Mitchell, 2006). We found no evidence of nematodes, other parasitic worms, or eggs in the fecal material. Composition of the fecalith comprised primarily indigestible matter, such as hair from small mammal prey and other unidentified material which may have included vegetable matter from the gut of its prey. As the snake appeared to be healthy, we did not assess other health parameters.

We held the snake in the laboratory without food until normal defecation indicated restored bowel function and healing sufficient for transmitter implantation and release to the wild. The snake produced a normal fecal bolus devoid of blood on 10 May, which was 19 days after the surgery. At this point, we surgically implanted a transmitter (Reinert and Cundall, 1982; Hardy and Greene, 1999; Hardy and Greene, 2000). Methods for anesthesia, access to the peritoneal space, and skin closure were identical to the previous surgery except that the snake was placed in right lateral recumbency and a 3-cm incision was made approximately 70 cm (two-thirds the length of the snake) from the head on the right side of the snake. We chose the incision site in part to avoid negatively impacting the prior surgical wound. This second surgery was uneventful and the snake recovered without complications. We released the snake near the site of capture the following morning (11 May).

Subsequent to release, we tracked the snake's movements via radio-telemetry for seven months. We obtained 45 location fixes during this period, with no abnormal movement patterns detected (c.f. Brown et al., 2008; Dugan et al., 2008). On 28 June, we observed a large intact shed skin extending out of a burrow occupied by the snake, suggesting she was shedding normally. The last location fix was obtained on 1 December 2010. Subsequent attempts to obtain a location fix failed because we were unable to detect a signal from the transmitter. The most likely explanation for this was transmitter failure, though a predation event could have moved the transmitter beyond detection range.

Discussion

Few reports exist of gastrointestinal blockage in snakes. Most literature records involve foreign body obstructions in the gastrointestinal tracts of the black ratsnake (Smith, 1953; Jacobson et al., 1980; Zwart et al., 1986; Souza et al., 2004; Adams and Sleeman, 2005), though partial obstruction due to cancerous tumor was reported in a cornsnake (*Pantherophis guttatus*, formerly *Elaphe guttata*; Latimer and Rich, 1998), and an obstruction potentially caused by a fecalith was reported in a gopher snake (*Pituophis melanolucus*; Jessup, 1980). Outside the literature, a case of apparent fecalith-induced gastrointestional obstruction was reported in a Burmese python (*Python molurus bivittatus*) at the Long Beach Animal Hospital website (Long Beach Animal Hospital, undated).

Considering the hardened nature of the obstruction and its large size, we believe the snake would not have survived without surgical intervention. We caught the obstruction early, as there was no evidence of body dehydration or emaciation, symptoms typical of other snake intestinal obstruction cases in the literature. Survival of the snake for at least seven months (until presumed transmitter failure) while exhibiting normal behaviors (c.f. Brown et al., 2008; Dugan et al., 2008) suggests that the snake was healthy.

Stout, heavy-bodied terrestrial vipers have a normal propensity to accumulate fecal material for many months, in some cases exceeding more than a year (Lillywhite et al., 2002). Fecal retention is more protracted with larger body size and infrequent meals (Lillywhite et al., 2002), which are characteristics of *C. ruber* (Dugan and Hayes, 2012). Lillywhite et al. (2002) suggested that fecal retention helps anchor the posterior body to facilitate extension and acceleration of the forebody during a strike, and anchoring the body after capture of a large prey item (the adaptive ballast hypothesis). Arboreal vipers, in contrast, retain fecal material for only days or weeks following a meal, ostensibly to shorten the duration of overloading their bodies during locomotion and to decrease the energy expenditure required to counteract gravity (Lillywhite et al., 2002; Tsai et al., 2008). Regardless of the adaptive value of long-term fecal retention (if any), our observation suggests that it may pose an overlooked risk of fecalith development and intestinal obstruction in stout, heavy-bodied terrestrial vipers.

In this case, the snake was captured during spring mating season, which immediately followed winter brumation (≈hibernation). Low temperatures reduce gastrointestinal motility in reptiles (Naulleau, 1983; Diaz-Figueroa and Mitchell, 2006), and snakes during brumation may experience substantial water loss (Costanzo, 1989). These factors may place temperate snakes at greater risk for fecalith development and gastrointestinal obstruction than tropical snakes. Drought, which has plagued the southwestern United States (including our study site) in the recent decade, and is projected to feature prominently in the next century (MacDonald, 2010), may exacerbate the risk of fecalith development if it negatively affects the snake's hydration state. A diet composed largely of small mammals with indigestible hairs that accumulate in the feces (Dugan and Hayes, 2012) may also predispose fecalith development. We suggest that field researchers pay more attention to the frequency of palpable fecaliths in free-ranging snakes, especially with regard to taxonomic, climate, seasonal, and dietary variables.

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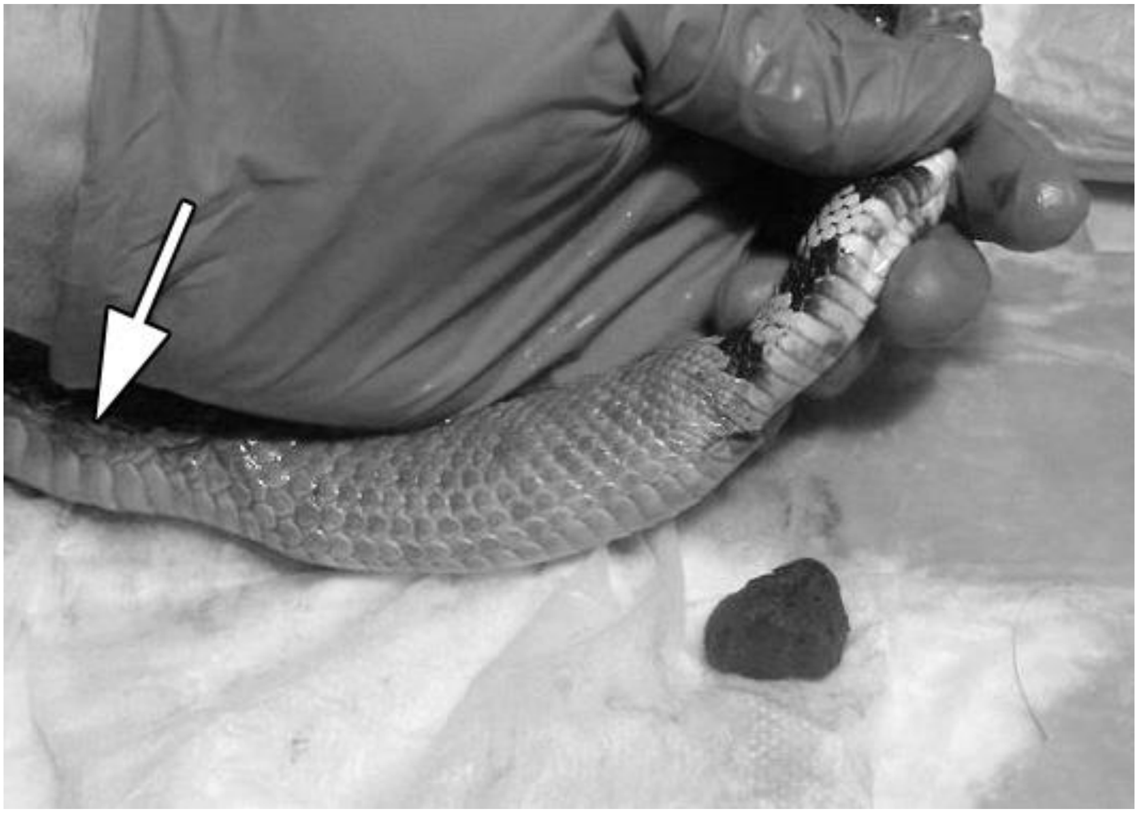

Figure 1. Hardened fecalith and the posterior end of the wild, female red diamond rattlesnake (*Crotalus ruber*) it was removed from. Arrow shows the position of the fecalith prior to its removal. Photograph taken immediately after closure of the surgical incision and just before the snake was placed in a heated terrarium for recovery.