EVALUATING CONSERVATION STRATEGIES FOR A THREATENED POPULATION OF GRAY RATSNAKES (*PANTHEROPHIS SPILOIDES*)

by

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Abstract

Wildlife populations across the globe are declining due to the effects of increasing anthropogenic activities. Among the most vulnerable taxa are snakes, which face several threats including road mortality and habitat loss. To combat such threats, several different conservation techniques have been implemented. Roadside barrier fencing is designed to reduce adult mortality by preventing snakes from accessing the road, while artificial nesting sites serve to increase recruitment. Despite their growing use, however, these strategies are seldom rigorously tested before or after implementation. In this study, I examined the effectiveness of roadside barrier fencing and artificial nest boxes for gray ratsnakes (*Pantherophis spiloides*), a species at risk in Canada. The goals of my research were to (1) determine the fencing design that prevents gray ratsnakes from successfully climbing over, and (2) determine the environmental variables that influence the use of nest boxes by gray ratsnakes. To do this, I captured and placed gray ratsnakes within fencing enclosures composed of different heights, materials, and shapes commonly used in roadside barrier fencing. I then measured whether or not snakes were able to escape, as well as different behavioral responses. I also placed nest boxes throughout various habitats and monitored their environmental conditions throughout the incubation period before checking them for snake eggs. My study revealed that fence material played a significant role in whether or not a snake could climb it, and found a significant interaction between height and shape on snake climbing success. Further, I found that snakes were less willing to climb fencing that was higher and made out of hardware cloth than vinyl sheeting. I also found a near-significant relationship between whether or not a nest box was used and a combination of internal temperature, moisture, and canopy cover. Nest boxes that were used featured higher internal temperatures, moisture, and mid-range canopy covers; however further investigation is needed due to lack of power given the small sample size. My study highlights the importance of identifying and rigorously investigating knowledge gaps surrounding conservation strategies, to maximize their effectiveness and avoid wasting already-limited conservation funding.

Co-authorship

Chapter 2: Keeping snakes off roads: A test of the effectiveness of barrier fencing incorporating animal behaviour and morphology

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Originally submitted June 20, 2020. Will be re-submitted with revisions October 2020. The article was conceived by Stephen C. Lougheed, Jacqueline D. Litzgus, Patrick C. Weatherhead, and me. Meghan Ewing and I performed the experiment. I conducted the analyses. I researched and wrote the first draft of the manuscript, which was then edited by Stephen C. Lougheed, Jacqueline D. Litzgus, and Patrick C. Weatherhead.

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“From the very beginning of the world, the other species were a lifeboat for the people. Now, we must be theirs.” – Robin Wall Kimmerer, Braiding Sweetgrass

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List of Abbreviations

ANOVA: Analysis of variance

COSEWIC: Committee on the Status of Endangered Wildlife in Canada

HDPE: High-density polyethylene

IUCN: International Union for Conservation of Nature

LGSC: Leed-Grenville stewardship council

MTO: Ontario Ministry of Transportation

OMNR: Ontario Ministry of Natural Resources

OMNRF: Ontario Ministry of Natural Resources and Forestry

PCA: Principal component analysis

PC1: First principal component

PIT: Passive integrated transponder

QUBS: Queen’s University Biological Station

SE: Standard error

SVL: Snout-to-vent length

Chapter 1

General Introduction

Many wildlife species across the globe are experiencing population declines, largely due to the effects of increasing anthropogenic activity (Butchart et al., 2010; Hoffman et al., 2010, WWF, 2016). Snakes are among the vertebrate groups most at risk; 12% of assessed snakes species are listed as threatened by the IUCN, with a further 62% of global reptile species awaiting assessment (Böhm et al., 2013). In Canada alone, 77% of reptile species are considered at risk and experiencing population declines, with several species already extirpated (Lesbarrères et al., 2014). Such declines are due to a variety of direct and indirect threats such as road mortality, persecution, habitat loss and fragmentation, climate change, subsidized predators, and emerging infectious diseases (Gibbons et al., 2000; Seburn and Seburn, 2000). These threats lead to population declines by either decreasing the number of breeding adults, limiting population recruitment, or both (Seburn and Seburn, 2000). While the effects of direct threats such as road mortality and persecution are relatively straightforward as they lead to the immediate removal of individuals from a population, the effects of indirect threats are often not as clear. Studies surrounding habitat loss and fragmentation often focus on loss of connectivity or area, and how this affects the movement and genetic variation at the population and species-level (Crosby et al., 2009; Row et al., 2012). However, habitat loss and fragmentation can also lead to a reduction in the availability of key resources within an ecosystem such as suitable nesting, gestation or hibernation sites, which in turn can limit a population's potential carrying capacity within that ecosystem (Aitken and Martin, 2011; Johnson et al., 2016; Kingsbury and Coppola, 2000; Philpott and Foster, 2005). Such threats can prove even more substantial for species with a late age of sexual maturity and low reproductive rate, where even a slight increase in adult mortality or reduction in adult recruitment potential can have a negative impact on population persistence (Brooks et al. 1991; Congdon et al., 1993).

Several different conservation techniques and management practices have been designed and implemented to combat these threats and maintain healthy populations of species at risk. For reptile species at risk, such practices include roadside barrier fencing, habitat protection, wildlife corridors, artificial hibernacula, basking, gestation and nesting sites, and captive breeding and head-starting (Ananjeva et al., 2015; Baxter-Gilbert et al., 2015; Choquette et al., 2020; Johnson et al., 2016; Paterson et al., 2013; Zappalorti et al., 2014). Strategies such as the placement of exclusion fencing along roadsides are designed to reduce adult mortality by diminishing wildlife access to roads (Dodd Jr. et al., 2004; Woltz et al., 2008). Other strategies, such as the creation of artificial nesting sites, serve to increase recruitment by subsidizing egg and juvenile survival (Buhlmann and Osborn, 2011; Marchand and Litvaitis, 2004; Paterson et al. 2013). Despite their growing use, however, these strategies are not always tested before implementation and in some cases either fail to alleviate or even exacerbate threats (Brooks et al., 2006; Podloucky, 1989). For example, following the construction of exclusion fencing along a busy highway, Baxter-Gilbert et al. (2015) found no difference in reptile abundance on the road, likely due to frequent rips in the fencing material. In addition, they observed increased snake and turtle road mortality, as animals that were able to access the road became trapped by the fencing therefore prolonging their exposure to traffic. While the implementation of artificial nest sites has been shown to benefit several species at risk by increasing offspring fitness and survival, studies have also shown that they can also act as ecological traps (Demeyrier et al. 2016; Paterson et al. 2013). For example, Klein et al. (2007) found that barn owl (*Tyto alba*) owlets developing in external nest-boxes had significantly lower survival than those hatched in a more “natural” environment of church towers. To avoid such situations, factors such as animal behaviour, structural design, and location need to be thoroughly tested and their effects quantified before mitigation is implemented (Baxter-Gilbert et al., 2015; Podloucky, 1989).

1.1 Gray Ratsnake (*Pantherophis spiloides*)

The gray ratsnake (*Pantherophis spiloides*) is the longest snake species in Canada, averaging 1.5 to 1.8 meters in total length at maturity (Blouin-Demers et al., 2002; Fig.1). The gray ratsnake is distributed across much of the eastern and central United States, continuing North from the Gulf of Mexico to Southern Ontario, and from Texas to the Atlantic coastline (COSEWIC, 2018). This semi-arboreal species is typically associated with woodland, forest edge, and field habitats (Ernst and Ernst, 2009). Home range size differs between the sexes, with males having an average range of 5.57 ha and females having an average range of 1.41 ha (Weatherhead and Hoysak, 1989). In eastern Ontario, ratsnakes overwinter in hibernacula, and begin to emerge in late April before moving to their respective home ranges (Blouin-Demers et al., 2000). In Canada, ratsnakes typically mate between late May and mid-June (COSEWIC, 2018). Most females reach sexual maturity at around 9.7 years of age and lay clutch sizes of 10-15 eggs in late June to early August, approximately every 2-3 years (Blouin-Demers et al., 2002; Blouin-Demers et al., 2005; COSEWIC, 2018).

In Canada, the gray ratsnake is confined to two geographically separate regions within the province of Ontario (COSEWIC, 2018). The Great Lakes/St. Lawrence population, at the northern limit of the species range, is associated with the Frontenac Axis and has been designated as Threatened (Blouin-Demers et al. 2002; COSEWIC, 2018). One of the significant threats faced by this population is road mortality, due to a combination of relatively large individual home ranges and a dense road network throughout the population range (Weatherhead and Hoysak, 1989). On a 10 km stretch of road, Row et al. (2007) documented an average of 6 gray ratsnakes killed by vehicles per year. Further, a population viability analysis indicated that as few as 3 adult females killed on roads each year raised the extinction probability of this local population to 90% in 500 years (Row et al., 2007). Another significant threat affecting the Great Lakes/St. Lawrence population is the loss and/or fragmentation of suitable habitat, which can have a negative impact on nesting success and behaviour (COSEWIC, 2018; Ontario Ministry of Natural Resources, 2005; Prior and Weatherhead, 2000). Gray ratsnakes nest in standing snags, stumps, logs, or compost piles, and show high degrees of both communal nesting and nest site philopatry (Blouin-Demers et al., 2004; COSEWIC, 2018). Similar to other colubrid species, female gray ratsnakes select nest sites based on factors such as the temperature and humidity within the nest, with a preference for higher humidity levels and temperatures of around 31°C (Blouin-Demers et al., 2004; Burger and Zappalorti, 1992; COSEWIC, 2018; Löwenborg et al., 2012; Plummer, 1990). By selecting these environmental conditions, ratsnake populations realize improved hatching success and offspring fitness (Blouin-Demers et al., 2004). Through the alteration or loss of habitat used by gray ratsnakes, the number of potential nest sites is reduced, which can in turn lead to fewer successful nests and therefore reduced levels of recruitment (Swain and Smith, 1978).

1.2. Thesis objectives

The goal of my thesis was to evaluate two conservation strategies for gray ratsnakes, roadside barrier fencing and artificial nest sites. In order to reduce road mortality of snakes in

Ontario, various types of roadside barrier fencing for snakes have been recommended that feature different heights, materials, and shapes depending on species (OMNRF). However, due to a lack of research into the subject, these recommendations are based off of anecdotal evidence alone. Although it would be ideal to test the response of different snake species to different types of barrier fencing, this would be difficult to achieve given the sample sizes, time, and funding required. Given that gray ratsnakes are the longest snake species in Ontario and excellent climbers (Mullin and Cooper, 2002), fencing that is effective for ratsnakes should be effective for all other snake species in the province. To address this knowledge gap, the goals of my second chapter were to 1) Quantify how fencing height, material, and shape interact to influence probability of climbing over the fencing, 2) Identify the fencing design that may best function as a barrier to ratsnakes, and 3) Investigate the effect of fencing design on ratsnake behaviour including number of climbing attempts and time required to climb over the fencing. Like roadside barrier fencing for snakes, artificial nest boxes for gray ratsnakes have been widely implemented with few studies investigating their efficacy. While some ratsnake nest boxes are used by multiple females over several years, other nest boxes have not been used at all (OMNR, 2005; Smith, 2019; LGSC pers. comm.; S. Marks pers. comm.). The goal of my third chapter was to identify which environmental variables determine the use of gray ratsnake nest boxes. Because ratsnakes select nest sites based on substrate temperature and moisture content, I predict that these factors will play the largest role in determining the use of nest boxes by gray ratsnakes. Further, because such factors are dependent on environmental conditions outside of the nest box (Shorohova and Kapitsa, 2014), I expect that habitat features such as canopy cover would also play a significant role in determining use.

Figure 1. Gray ratsnake (*Patherophis spiloides*) from the Frontenac Axis population in Ontario, Canada (Photographer: Steven J. Kell, 2018).

Figure 2. Distribution of *Pantherophis alleghaniensis, obsoleta,* and *spiloides* (formally *Elaphe*). The red circle highlights the Frontenac Axis population of *P. spiloides*. Map modified from COSEWIC, 2018 (originally modified from Burbrink, 2001).

Chapter 2

Keeping snakes off roads: a test of the effectiveness of barrier fencing incorporating animal behavior and morphology

2.1 Introduction

Many wildlife species are experiencing population declines, largely due to the increasing effects of human activities (Butchart et al., 2010; Hoffman et al., 2010; WWF, 2016). A significant threat to many terrestrial species is road traffic, which is estimated to cause the deaths of millions of vertebrates each year (Rytwinski et al., 2016). These deaths lead to population declines by decreasing the number of breeding adults, limiting recruitment, or both (Seburn and Seburn, 2000). Consequences can be even more pronounced for species with a late age of sexual maturity and low reproductive rate, for which even slight increases in adult mortality can have a negative impact on population persistence (Brooks et al., 1991; Congdon et al., 1993; Row et al., 2007). To counter the effects of road traffic alone, over 40 types of mitigation strategies have been described, ranging from signs warning drivers to the presence of wildlife to exclusion fencing and ecopassages (OMNRF, 2016; Rytwinski et al., 2016).

Despite the numerous strategies proposed for diminishing road mortality, surprisingly few were tested before implementation, and in some cases failed to alleviate or even increased road mortality (Brooks et al., 2006; Baxter-Gilbert et al., 2015; Jones et al., 2019). A meta-analysis by Rytwinski et al. (2016) found exclusion fencing to be the most effective mitigation strategy to reduce road mortality, diminishing roadkill by an average of 54% across studies and taxa. The extent to which mitigation strategies reduced road mortality varied markedly, likely attributable to underlying factors like differences in interspecific behaviour and morphology, structural design, and study design (Baxter-Gilbert et al., 2015; Rytwinski et al., 2016). Mitigation studies were significantly biased towards large mammals, with other taxa such as small mammals, reptiles, amphibians, and birds underrepresented (Rytwinski et al., 2016). This is surprising given that reptiles and amphibians are among the vertebrate groups most at risk of extinction (Gibbons et al., 2000; Hayes et al., 2010; Böhm et al., 2013), and also among those most affected by road mortality (Andrews et al., 2008; Brehme et al., 2018). Additionally, because road mitigation strategies are often focused on relatively broad taxonomic groups (e.g. small mammals, anurans), interspecific differences in behaviour, morphology, and life-history traits among these groups are often not considered when assessing mitigation effectiveness (Woltz et al., 2008; Hamer et al., 2014). Also, although roadside barrier fencing can vary in height, material, shape, and length, most studies testing the effectiveness of mitigation fencing fail to adequately describe the attributes of the fencing used (Rytwinski et al., 2016).

To improve the success of conservation strategies such as exclusion fencing, it is clear that we must consider animal behaviour and structural design, and that these must be studied in both controlled and field settings before implementation on a broad scale (Woltz et al., 2008; Baxter-Gilbert et al., 2015; Rytwinski et al., 2015). I examined the potential of various barrier fencing designs for keeping gray ratsnakes (*Pantherophis spiloides*) off roads. Gray ratsnakes are listed as “Threatened” in Ontario where I conducted the study, due in part to the effects of road mortality (Row et al., 2007). Although it would have been ideal to test all co-occurring snake species given that they too are affected by road mortality, this would have been difficult to achieve given the sample sizes, time, and funding required. However, by choosing a focal species with the greatest potential to climb fencing, one could assume that the effectiveness of a given fence design for the focal species would be at least as effective for all other local snake species.

Ratsnakes are among the longest snake species in North America and are excellent climbers (Mullin and Cooper, 2002). Both of these attributes increase the risk of road mortality and decrease barrier fencing effectiveness for snakes (Dodd Jr. et al., 2004; Brehme et al., 2018). Thus, by using gray ratsnakes as our focal species, I assumed that our results would apply to most North American snakes.

My goals were to 1) Identify the fencing design that may best function as a barrier to ratsnakes, 2) Quantify how fencing height, material, and shape interact to influence probability of climbing over the fencing, and 3) Investigate the effect of fencing design on ratsnake behaviour including number of climbing attempts and time required to climb over the fencing.

2.2 Methods

I conducted experiments from 1 May – 30 August, 2019 at the Queen's University Biological Station (QUBS) near Elgin, ON, Canada (44.5675° N, 76.3245° W). In this study, I used fencing composed of two materials (hardware cloth and vinyl sheeting), two heights (60 and 100 cm), and two different shapes (straight and featuring a lip), resulting in 8 different fence configurations. The various material, height, and shape combinations were based on suggestions provided by the Ontario Ministry of Natural Resources and Forestry (OMNRF, 2016) for the snake species found in the study area. The hardware cloth (i.e. wire mesh) I used featured 6.3 mm gaps and was composed of double zinc-coated wire. The vinyl sheeting I used was composed of black HDPE-2 plastic with a thickness of 2 mm. Although both concrete and aluminum sheeting are also suggested for use as exclusion fencing (OMNRF, 2016), I did not test these materials in our study due to logistical, budget, and time constraints. Further, while the OMNRF also suggests using 2 m-high fencing for ratsnakes, I did not test this height based on preliminary trials in which 1 m fencing showed success in stopping ratsnakes from climbing over the fencing, as well as conversations with members of the Ontario Ministry of Transportation (MTO) who suggested that its use along roadsides would be highly unlikely due to appearance and cost. The lips (i.e. overhangs) present on some fencing combinations were 10 cm in length and extended 90° from the top of the fencing towards the inside of the enclosure.

I designed the testing enclosure so that fencing material could be easily affixed and removed as needed (Fig. 3). The enclosure was constructed using four wooden fence posts arranged in a 2m diameter circle on level ground (Woltz et al., 2008) to ensure that even the longest snakes would not be able to anchor or push themselves against a wall while attempting to climb the opposing wall. Fencing was attached to the posts using three 6 mm carriage bolts per post, with the round head of each bolt positioned on the inside of the enclosure to ensure that the snakes could not use them as leverage during escape attempts. The vertical seam created where both ends of the fencing overlapped was positioned in line with the bolts on one of the fence posts to ensure that the fencing was flush and there were no gaps through which snakes could escape. I opted not to bevel or smooth the seam because we considered that this was unlikely to be done when fencing is installed along roadways. The bottom of the fencing was buried 10 cm deep so that no snakes escaped by crawling underneath (thus, a fence that is 100 cm high extended an additional 10 cm underground).

Snakes were captured opportunistically on the QUBS property, and held for less than 72 hours. Individuals not previously marked (Weatherhead and Blouin-Demers, 2004) were implanted with a single passive integrated transponder (PIT) tag (12.5mm Biomark APT12, Biomark, Boise, ID) shortly after capture to avoid resampling. The PIT tags were implanted subcutaneously slightly anterior to the cloaca on the flank, and only individuals with a snout-to-vent length (SVL) of 30 cm or greater were tagged and tested (King et al., 2016). I then allowed the snakes to recover for 12 hours by placing them individually in large plastic tote bins (50x33x33 cm) before testing.

Prior to the start of each trial, individuals were allowed to acclimatize for 5 minutes in a small, overturned plastic tote bin (35x23x15 cm) within the enclosure (Andrews and Gibbons, 2005; Woltz et al. 2008; Baxter-Gilbert et al., 2015; Colley et al., 2017). To limit interaction between the snake and observer, the container was slowly lifted from 2 m away from the testing enclosure using a rope and pulley system (Andrews and Gibbons, 2005; Woltz et al., 2008; Baxter-Gilbert et al., 2015; Colley et al., 2017). Each trial lasted 30 minutes (Baxter-Gilbert et al., 2015), or until the snake escaped from the enclosure. All observations were made from a camouflaged blind 5 m from the testing arena to further minimize interaction between the subject and observer. All trials were recorded using a GoPro Hero 4 camera fixed directly above the testing enclosure for later review. The number of climbing attempts during each trial was recorded, with an individual actively climbing up the side of the fencing before either escaping or falling back down to the ground representing an attempt. I timed each trial to determine time until escape, which was defined as the time from when the acclimatization bin was lifted to the time when the snake’s head reached over the top of the fencing. If a snake did not escape the enclosure after 30 min, the trial ended and the snake was removed from the enclosure. Climbing attempts per minute were obtained by dividing the number of climbing attempts observed in each trial by the time until escape.

Before and after each trial I recorded the temperature of the ground using a pocket weather station (Kestrel 5500 Weather Meter, Kestrel Meters, Minneapolis, MN), because temperature is known to influence snake behaviour (Heckrotte, 1962; Goode and Duvall, 1989;

Brodie III and Russell, 1999). After the trial I recorded the SVL and total length of each snake because size is likely to have a strong influence on a snake's ability to climb (Jackson, 1976; Lillywhite et al., 2000). I recorded lengths as the mean of three measurements made using a flexible measuring tape placed along the side of the snake's body (Blouin-Demers, 2003). This was done only after each individual was finished their respective trials to avoid influencing behaviour during testing (Colley et al., 2017).

To obtain an adequate sample size given the relatively small population size, I tested some individual snakes more than once. Each individual was exposed to a maximum of four fencing combinations, with most individuals being tested only once or twice. Between trials, I released snakes where we had initially captured them. Therefore, only individuals that were recaptured a minimum of one week after a previous capture were retested. Each individual was tested only once per fencing arrangement to minimize changes in behaviour due to the influence of repeated stimuli (Martin and Bateson, 1986). Further, because experimental returns can depreciate through the repeated testing of subjects (Martin and Bateson, 1986), I used a random number generator program (in R) to randomize the order of fencing arrangements to which snakes were exposed.

I used R 3.5.2 (R Core Team, 2020) for all analyses. I confirmed that the distribution of both SVL and ground temperature were normal using Shapiro-Wilks tests to confirm there were no biases in the individuals selected for testing or conditions during testing. I used the package lme4 (Bates et al., 2014) to analyse three linear mixed-effects models to compare escape, climbing attempts per minute, and time until escape, respectively, against fencing material, fencing height, fencing shape, SVL, ground temperature, and individual tested. The fence material, height, and shape, SVL, and ground temperature were considered fixed effects, while the individual tested was considered a random effect. Interaction effects were only considered between fence material, height, and shape. Finally, the analysis for time to escape used only those trials that led to a successful escape.

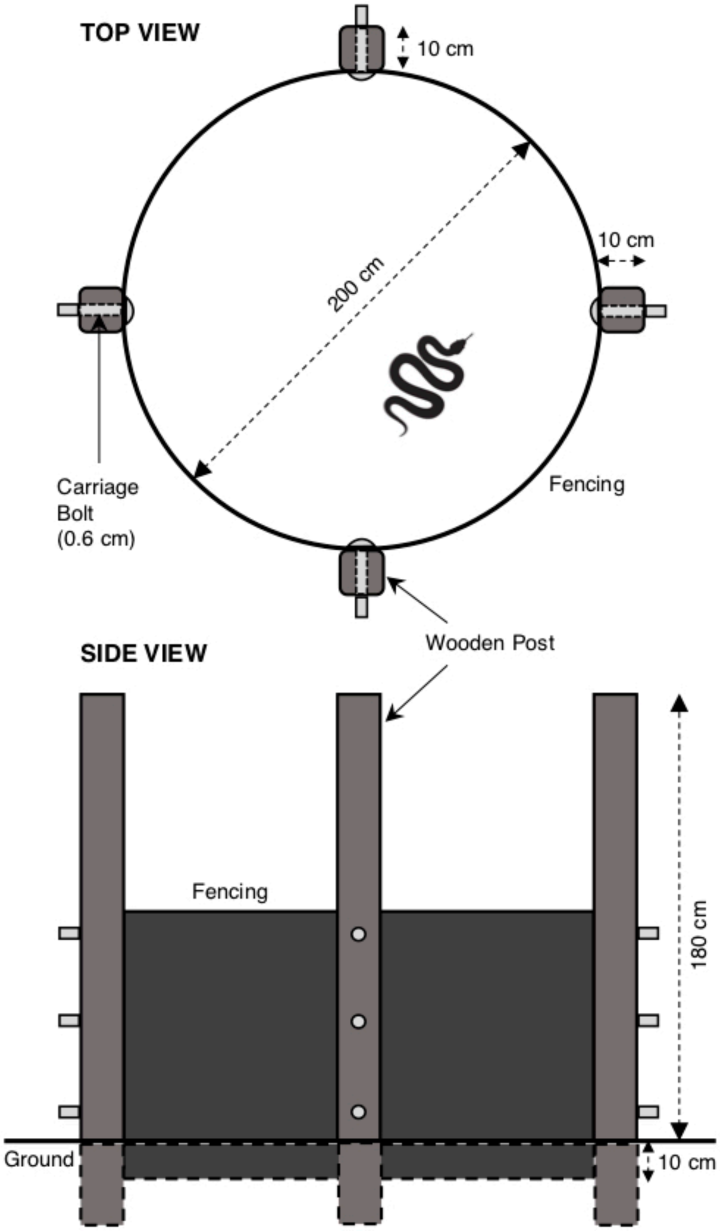

Figure 3. Schematic of the fencing configuration used in this study. Not to scale.

2.3 Results

I conducted a total of 88 trials, with a minimum of 10 per fencing arrangement. I used 56 ratsnakes in total, ranging in SVL from 743 to 1557 mm (mean = 1114 ± 20 SE). The average ground temperature during testing ranged from 18 to 37.1°C (mean = 26.6 ± 0.5 SE). Most snakes tested began to explore the testing arena within 1 minute after the holding container was lifted, and all began moving within 6 minutes. Moreover, all of the snakes remained active throughout the trials exploring and attempting to escape, except for one individual that stopped moving after 15 minutes and basked for the remainder of the trial.

Snakes were most successful at climbing over the 60 cm vinyl sheeting fencing enclosures (with and without a lip) with a success rate of 100% (n=10/10), and were the least successful climbing over the 100 cm hardware cloth fencing enclosure with a lip with a success rate of 6.7% (1/15; Fig. 4). Snakes were more likely to escape from fencing that was made of vinyl sheeting ($t_{87} = 2.30$, $p = 0.025$; Fig. 4), and longer snakes were more successful escaping than shorter individuals ($t_{87} = 2.05$, $p = 0.045$). I found no significant effect of ground temperature on snake escape success ($t_{87} = -0.20$, $p = 0.84$). However, I did find a significant interaction effect between fence height and fence shape on the likelihood of escape ($t_{87} = -2.25$, $p = 0.028$). Although the presence of a lip reduced the likelihood of escape in both the 60 cm and 100 cm fencing designs, the observed difference was greater in the shorter fencing (Fig. 5).

Fencing material ($t_{86} = 4.78$, $p < 0.001$), height ($t_{86} = -2.43$, $p = 0.017$), and ground temperature ($t_{86} = 3.90$, $p < 0.001$) had significant effects on the number of climbing attempts made per minute (Fig. 6), but neither fence shape ($t_{86} = -0.59$, $p = 0.56$) nor SVL ($t_{86} = 0.93$, $p = 0.359$) had a significant effect. I found no significant interaction effects among any of the fence variables and climbing attempts per minute. Snakes climbed more often when presented with vinyl sheeting fencing and/or shorter fencing (Fig. 6), and when the ground was warmer. Individuals made the greatest number of climbing attempts per minute when 60 cm vinyl sheet fencing was used (mean = 1.44 ± 0.19 SE), and made the fewest attempts when 100 cm hardware cloth fencing was used (mean = 0.59 ± 0.07 SE; Fig. 6).

For those individuals that managed to climb over the fencing ($n = 44$), I found a significant relationship between the time until escape and both the height of the fencing ($t_{43} = 3.35$, $p = 0.002$; Fig. 7) and ground temperature ($t_{43} = -4.53$, $p < 0.001$), but not the fencing material ($t_{43} = -1.27$, $p = 0.21$), fencing shape ($t_{43} = 0.67$, $p = 0.51$), or SVL ($t_{43} = 0.53$, $p = 0.60$). I found no significant interactions among any of the fence variables and time taken to climb over the fencing. Snakes took less time to climb over fencing that was shorter and at higher ground temperatures (Fig. 7). When presented with the 60 cm fencing, snakes were able to climb over within an average of 540 ± 61.7 s, whereas they took an average of 1030 ± 121 s when presented with the 100 cm fencing (Fig. 7). Some snakes tested against the vinyl sheeting used the vertical seam when climbing (25%, $n = 10/40$; Fig. 8). In contrast, no snakes tested against the hardware cloth used the vertical seam to climb ($n = 0/48$).

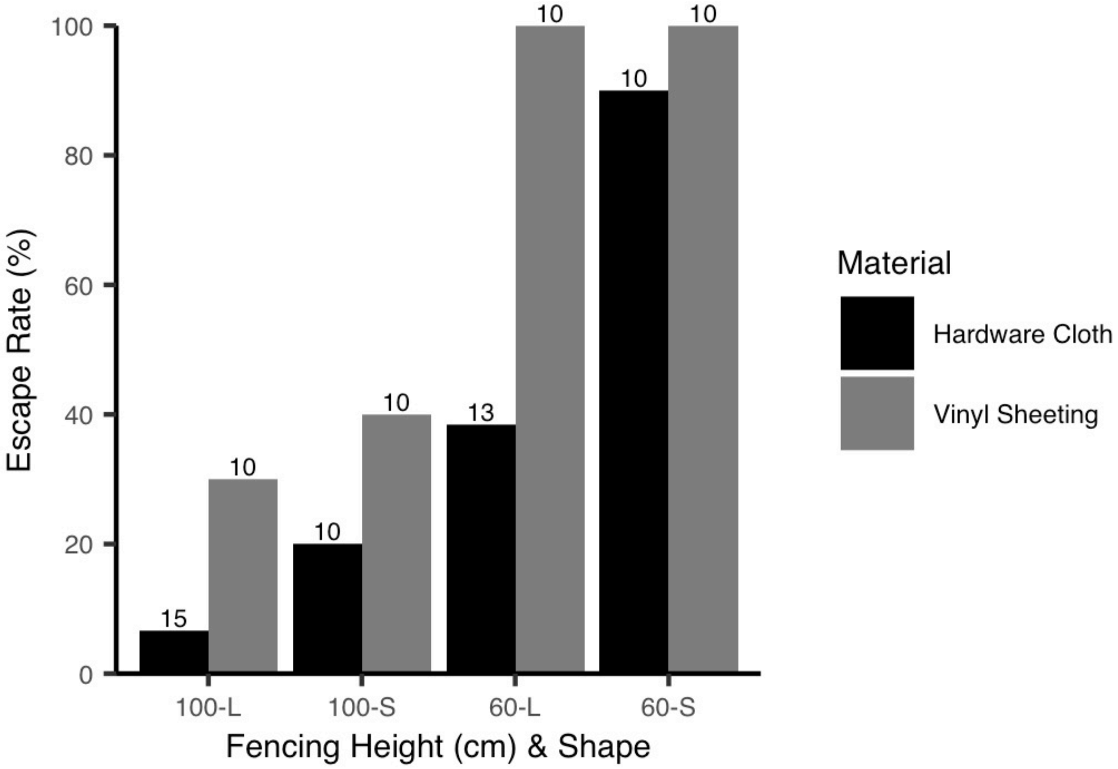

Figure 4. Escape rates of gray ratsnakes (*Pantherophis spiloides*) under different combinations of fencing materials. HC = Hardware Cloth, VS = Vinyl Sheeting, 60 = 60 cm height, 100 = 100 cm height, L = Lip, and S = Straight. The numbers at the top of the bars represent the sample size for each treatment.

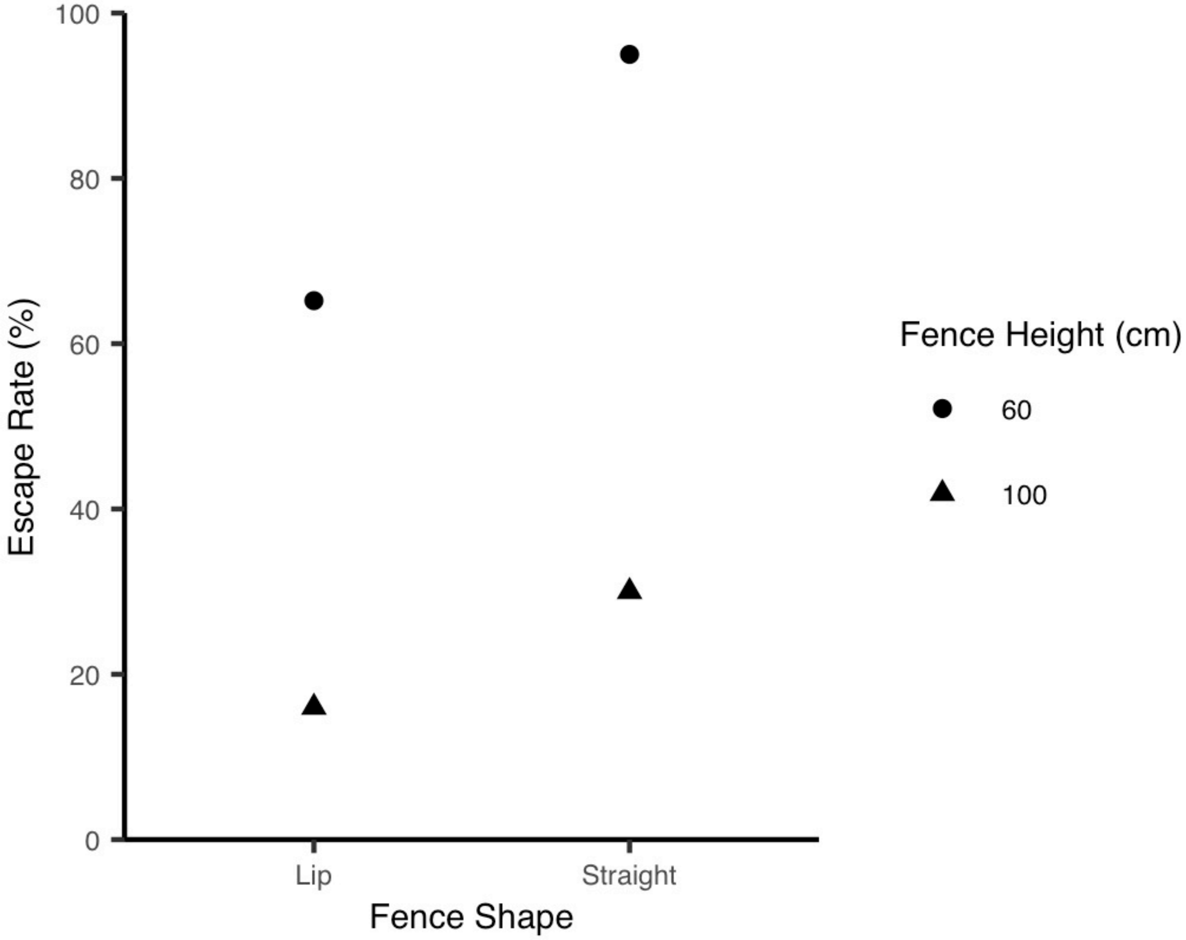

Figure 5. Interaction plot of the effect of fence height and shape on escape rate.

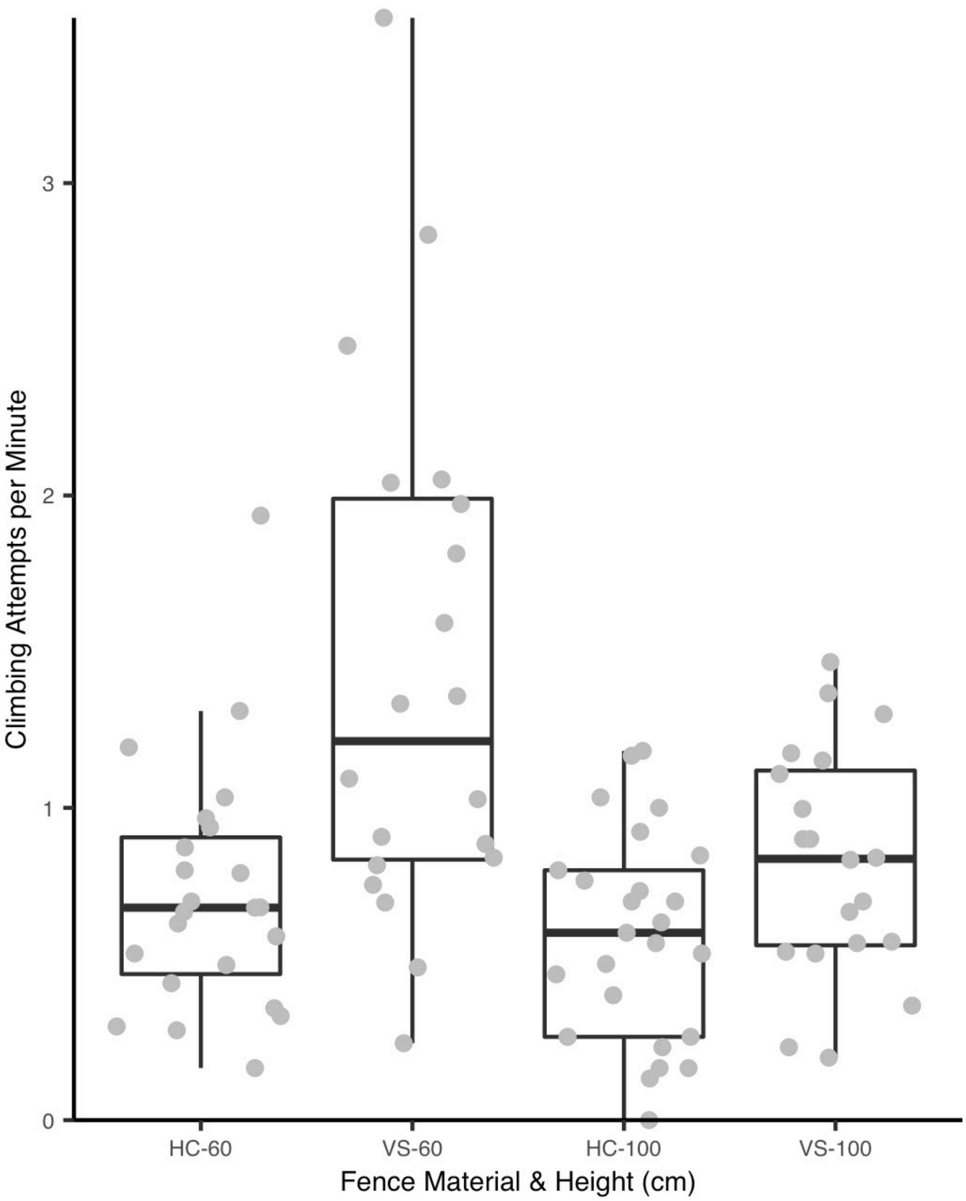

Figure 6. Number of climbing attempts made by gray ratsnakes (*Pantherophis spiloides*) under different combinations of fencing materials and heights. N = 23, 20, 25, 20 (respectively). The middle line of each box represents the median. The gray dots represent the individual data points for each fence combination, jittered so that all are visible.

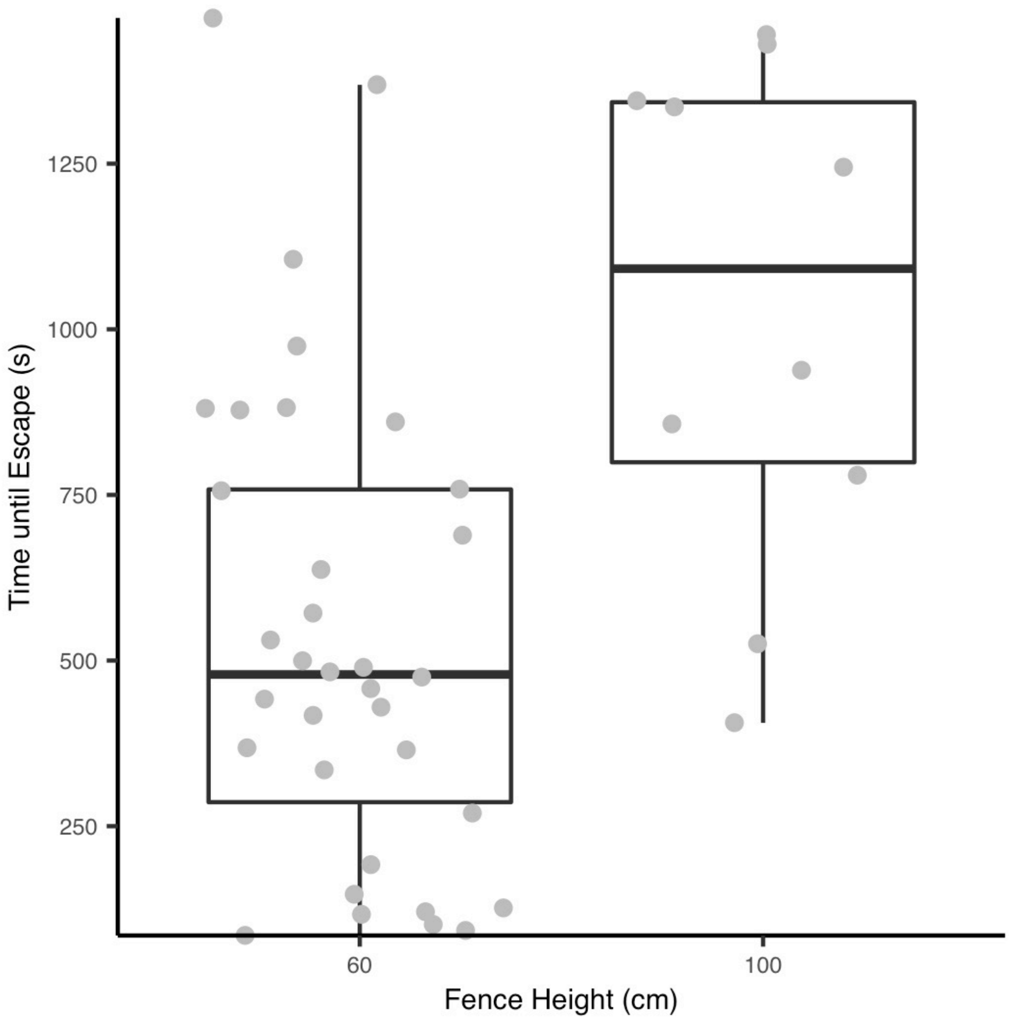

Figure 7. The time taken for gray ratsnakes (*Pantherophis spiloides*) to climb over fencing under different fence heights. N = 34, 10 (respectively). Other details as in Figure 6.

Figure 8. Gray ratsnake (*Pantherophis spiloides*) using the vertical seam of the fence during its escape from 100 cm vinyl sheeting with a lip.

2.4 Discussion

My goal was to test the effectiveness of different roadside barrier fencing designs for gray ratsnakes, the largest and most arboreal of nine snake species found in our region. The assumption of my study is that fencing that diminishes ratsnake road mortality will similarly be effective for other snake species, and likely for some other co-distributed taxa such as turtles and ranid frogs. I found the most effective fencing to be 100 cm high hardware cloth with a lip. Road mortality is a significant contributor to overall mortality in this regional population of ratsnakes (Row et al. 2007), and proper implementation of this fencing would likely have a significant impact on reducing its extinction probability. Further, while potentially not as durable as the concrete and aluminum fencing designs recommended by the OMNRF (2016), hardware cloth fencing would be significantly cheaper with regard to materials and time to construct and therefore has a much greater chance of implementation on a large scale.

My analyses indicate that the material, height, and shape of barrier fencing all contribute to variation in the success of ratsnakes in climbing over fencing. The height of the fencing played the largest role in the ratsnakes' ability to climb, with fewer ratsnakes climbing over the 100 cm fencing compared to the 60 cm fencing. I also observed an increase in the amount of time taken to climb over higher fencing. However, I do not know whether this is simply because it took the ratsnakes more climbing attempts before they successfully climbed over the fencing, or whether they were taking longer to explore the enclosure and look for other avenues of escape. The material used in the fencing also had a significant impact on the ratsnakes' ability to climb it, with fewer ratsnakes successful at climbing over hardware cloth fencing. This is contrary to other studies that have shown that ratsnakes have an easier time climbing rougher materials, which allows the ratsnakes to gain better purchase using their ventral musculature and scales, allowing them to climb higher (Mullin and Cooper, 2002). Instead, due to the transparency of the hardware cloth fencing versus the opaque vinyl sheeting, the ratsnakes spent less time trying to surmount the fencing (resulting in fewer climbing attempts per minute) and instead focused on attempting to push through the fencing. This is similar to the phenomena observed in captive reptiles, wherein they attempt to push, dig or claw through the vertical glass walls of the vivarium instead of focusing their effort on more probable escape routes elsewhere (Warwick et al., 2013). Moreover, several of the ratsnakes tested against the vinyl sheeting fencing used the edge of the vertical seam created where the ends of the fencing overlapped (~2.5 mm) to help balance themselves as they escaped, allowing them to climb higher and therefore increasing their odds of escaping the enclosure. In comparison, no individuals tested against hardware cloth used the vertical seam of the fencing during their climbing attempts.

I also found a significant interaction effect between fence height and fence shape on escape success. The presence or absence of a lip made a greater difference in the likelihood of escape for shorter fencing than for taller fencing. This was likely because most snakes were not long enough to reach the top of the 100 cm fencing, and therefore had no physical encounter with the lip. However, it also shows the importance of considering how the different fence variables interact with one another when designing roadside barrier fencing. While the presence of a lip might not make a significant difference when using taller fencing, it could significantly reduce road mortality when shorter fencing is used and therefore would be worth the extra investment. I found no significant relationship between the presence of a lip and either the number of climbing attempts or time to climb over the fencing. The difference in success rates between fencing with and without a lip appears to be due to the fact that the ratsnakes needed to lean back to a degree to overcome the overhang, reducing their ability to balance and therefore causing them to fall. Finally, my analyses also showed a significant relationship between the probability of escape and SVL, but not ground temperature. Although this result was expected based on previous studies that have found a relationship between the climbing ability and both the length and mass of snakes (Lillywhite et al., 2000; Pizzatto et al., 2007), it demonstrates the importance of considering morphological variability within a species when planning a conservation strategy. While the 100 cm hardware cloth fencing with a lip successfully prevented most of our ratsnakes from successfully climbing over, it may not prove as effective for gray ratsnake populations further south where individuals grow longer (DeGregorio et al., 2018).

Although I attempted to rigorously assess the influence of fencing design on the behavioural response of gray ratsnakes, our study had some limitations. My sample size was sufficient to examine the behavioural responses of ratsnakes to different combinations of fence attributes given the effect sizes observed, but may have been insufficient to detect interaction effects between fence material and other fence attributes. My analyses indicated no significant interaction effect between fence material and shape, although the difference in climbing success rate between straight and lipped fencing appears to be far greater when hardware cloth is used compared to vinyl sheeting (Fig. 3). Regardless, all fence attributes had a significant effect on climbing success and therefore all need to be considered. It is possible that the shape of fencing would make a greater difference when using hardware cloth compared to vinyl sheeting, and this should be tested further. Moreover, I was unable to determine the total distance the ratsnakes moved along the fence during the trials, and only tested them for 30 minutes due to animal care protocol stipulations. Although 30 min was more than enough time for snakes to climb over the fencing, it may not have been enough time to determine the distance a snake might travel along a fence deployed alongside a road. This is important information, as the distance between fence ends or ecopassages accompanied by roadside barrier fencing can be large, and can affect the success of mortality mitigating (Rytwinski et al., 2016). Further, my study only tested the response of individuals to commonly-used wildlife fencing materials; some of these materials such as hardware cloth do not last as long as other materials such as concrete or aluminum sheeting (Dodd et al., 2004). Therefore, while the initial cost of hardware cloth may be lower, the ongoing cost of maintaining a fence composed of such material may end up being more expensive in the long term (Baxter-Gilbert et al., 2015). Moreover, hardware cloth and vinyl sheeting may have other negative effects with the surrounding environment (e.g. toxic compounds in local soils). Finally, the short duration between PIT tag implantation and testing may have influenced success rates, although empirical studies suggest that PIT tags are unlikely to have marked effect on performance (e.g. Jemison et al., 1995).

My study clearly demonstrates that fencing material, height, and shape, as well as morphological variation within a species, strongly influence the success of roadside exclusion fencing and therefore all need to be considered in the design and deployment of exclusion fencing. I also show that behaviour is an important consideration (e.g. exploratory behaviour) in such conservation initiatives. Finally, my study underscores the importance of rigorously studying conservation strategies such as exclusion fencing in advance of implementation both to spend limited conservation funds judiciously but also to have greatest probability of success.

Chapter 3

Effectiveness of artificial nest sites for gray ratsnakes (*Pantherophis spiloides*)

3.1 Introduction

Ongoing and widespread habitat loss has led to the population declines of wildlife worldwide (Butchart et al., 2010; Hoffman et al., 2010; WWF, 2016). Although much attention is given to loss of connectivity or area, among the most limiting factors for many taxa is the availability of key features within an ecosystem such as suitable nesting, gestation or hibernation sites, which in turn can limit a population's potential carrying capacity within that ecosystem (Aitken and Martin, 2011; Johnson et al., 2016; Kingsbury and Coppola, 2000). As a result of the ongoing loss of nesting sites for many vertebrate species at risk, artificial nest sites have often been proposed and deployed in the hopes of increasing recruitment in local populations (Sutherland et al., 2014). Although this is often the primary goal for artificial nest sites for species at risk, they have also proven useful in other ways such as monitoring populations (e.g. estimating the number of laying females, hatching success), improving fitness of hatchlings, and decreasing mortality on roads by reducing migration distance to suitable nesting sites (Paterson et al., 2013). While the use of artificial nest sites has been studied for birds (Iezekiel et al., 2017; Marti et al., 1979; Munro and Rounds, 1985), small mammals (Catall et al., 2011; Madikiza et al., 2010), turtles (Paterson et al., 2013) and some insects (Gruber et al., 2011; Philpott and Foster, 2005), few studies have looked at their effectiveness for oviparous snake species.

Most oviparous snake species lay their eggs underground or beneath logs, rocks, or other debris (Blouin-Demers et al., 2004; Braz et al., 2008; Burger and Zappalorti, 1986). Females select nest sites based on environmental cues that influence the fitness of hatchlings in terms of their viability, size at hatching, and behaviour (Blouin-Demers et al., 2004; Brown and Shine, 2004). These environmental cues vary with latitude and habitat; in tropical climates oviparous snake nests are typically limited only by the water potential of the incubation substrate (Brown and Shine 2004). However, in temperate climates nest sites are limited by both hydric and thermal conditions given that ideal temperatures are not available year-round and incubation periods typically last only weeks to months (Brown and Shine, 2004; Löwenborg et al., 2010). Further, some temperate snake species nest communally and show a high degree of philopatry to nest sites, suggesting that suitable sites for nesting are a more limited resource at higher latitudes (Blouin-Demers et al., 2004; Burger and Zappalorti, 1992; Plummer, 1990; Löwenborg et al., 2010; Swain and Smith, 1978).

The gray ratsnake (*Pantherophis spiloides*) is a temperate, oviparous snake species whose northernmost range extends onto the Frontenac Arch, a Precambrian geological feature that dominates southeastern Ontario, Canada (COSEWIC, 2018). This species is typically associated with woodland, forest edge, and field habitats (Ernst and Ernst, 2009). In Canada, ratsnakes typically mate between late May and mid-June (COSEWIC, 2018). Females reach sexual maturity at around 9.7 years of age and lay clutch sizes of 10-15 eggs in late July to early August, approximately every 2-3 years (Blouin-Demers et al., 2002; Blouin-Demers et al., 2005; COSEWIC, 2018; Prior and Weatherhead 1996). Females oviposit within nest sites made of loose and decaying organic matter such as rotting tree stumps, the interior of hollow trees (both upright and fallen), and compost or leaf piles (Prior and Weatherhead 1996; Blouin-Demers et al. 2004). Although some females will nest individually, most ratsnake nests are communal (Blouin-Demers et al., 2004). The mean temperatures within nests can range from 22.2 to 31.8°C, with snakes displaying a preference for warmer and more humid nest sites (Blouin-Demers et al., 2004). The Frontenac population of gray ratsnakes is designated as Threatened in Canada due to several threats such as road mortality, persecution, and habitat loss and fragmentation (COSEWIC, 2018). While it has been argued that gray ratsnakes may have benefitted from habitat fragmentation because of their strong association with edge habitat (Blouin-Demers and Weatherhead, 2001), suitable natural nest sites for the Frontenac Axis population have declined as a result of the reduction of large dead and decaying trees where nest sites would naturally occur (Weatherhead and Madsen, 2009). This is due to both the decline in the extent of old-growth forest (most local forests are second-growth) and Dutch elm disease which has prevented American elms trees (*Ulmus americana*), a once common local tree, from growing to a diameter suitable enough to support the stable decomposition temperatures and moisture levels needed for successful gray ratsnake egg incubation (Leeds-Grenville stewardship council pers. comm.; Weatherhead and Madsen, 2009; Zambra et al., 2011).

To mitigate the loss of natural nest sites for gray ratsnakes, artificial nest sites were designed by the Leeds-Grenville stewardship council (LGSC) in the form of snake nest boxes that serve to simulate the natural nesting conditions of gray ratsnakes (LGSC, 2004). Since their initial design over a decade ago, these boxes have been widely used not only within the Frontenac Arch for gray ratsnakes but also in other regions of Ontario (Blott, 2017) and for other species such as the Eastern foxsnake (*Pantherophis gloydi*; Smith, 2019). While the nest boxes in some locations are used by numerous snakes on a yearly basis, other nest boxes have not been used since their initial deployment (OMNR, 2005; Smith, 2019; LGSC pers. comm.; S. Marks pers. comm.). Although previous preliminary studies have noted that environmental factors are likely the determining factors for the use and success of ratsnake nest boxes (OMNR, 2005; Smith, 2019), no formal study has yet been conducted with a sample size adequate enough to identify significant factors. In this study, I deployed 16 gray ratsnake nest boxes in various habitats to quantify the environmental variables that determine nest box use and success. Given the environmental conditions observed in the natural nest sites of gray ratsnakes, I expected that nest box temperature and moisture content would play the largest role in determining their use. Further, because these factors depend on some environmental conditions outside of the nest box (Shorohova and Kapitsa, 2014), I expected that habitat features such as canopy cover would also play a significant role in nest box use.

3.2 Methods

I conducted my study from 15 June – 15 October, 2019 within the Frontenac Arch region of Ontario, Canada (44.47276-44.62669° N, 76.43086-76.07552° W). Sixteen gray ratsnake nest boxes were constructed according to the plans provided by the LGSC (2004; Fig. 9). The nest boxes are square cubes (123 cm linear dimension), and consist of a white cedar frame covered in 5 x 5 cm wire mesh on all sides. This mesh size allows female snakes to pass through while preventing access by egg predators such as skunks and raccoons. I placed the nest boxes in field (n = 4), forest edge (n = 8), and forest (n = 4) habitats with a minimum distance between boxes of 500 m (Fig. 10). I defined forest edge habitat as the boundary between forest habitat and open habitat such as meadows maintained via annual mowing or water bodies. In areas where a road bisected suitable habitat, nest boxes were placed in pairs on either side with a minimum distance of 500 m from the road to discourage females from crossing the road while searching for a suitable nesting site. Most nest boxes were initially setup and placed in 2018; however one was placed in 2010 (DR01), one in 2014 (KB01), and two in 2017 (EL01, BO01). Nest boxes are refilled on a yearly basis in the middle of June with a locally-sourced mixture of 40% woodchips, 40% hay/straw, and 20% leaf litter (LGSC, 2004; OMNR, 2005). All nesting material that had not fully decomposed from the previous year was redistributed within the nest box, and the decomposed material was scattered throughout the area surrounding the nest box. I then made sure that all nesting material in each box was thoroughly mixed using a pitchfork.

To measure internal temperatures over time, I placed three temperature data loggers (Thermochron iButton -40°C to 85°C; Embedded Data Systems, Lawrenceburg, KY) in the center of each box after it had been filled with nesting material; one iButton on the surface of the nesting material (0 cm), one at a depth of 20 cm, and the third at a depth of 40 cm (Blouin-Demers et al., 2004). Prior to placement, the temperature data loggers were launched, water-proofed with a rubber coating (Plasti Dip®; Plasti Dip International, Blaine, MI), labeled with flagging tape, and attached to a length of fishing line for securement to the nest box. I set the data loggers to record the temperature once every two hours throughout the local gray ratsnake egg incubation period (late-July to early-October; Blouin-Demers et al., 2004; Prior and Weatherhead, 1996). I also took weekly measurements of nest box moisture content, canopy cover, air temperature, and air humidity throughout the incubation period to determine the effect of these variables on nest box use. Moisture content was measured using an Extech MO750 Soil Moisture Meter (Extech, Nashua, NH) at four locations in each nest box near the base and the mean was calculated each time. I collected four measurements to account for moisture variation resulting from dry pockets of hay/straw and wetter pockets of decaying matter. Measurements were always taken at the same location within each box to minimize the chance of disturbing/damaging any eggs that may have been present. I measured air temperature and humidity immediately adjacent to each box using a pocket weather station (Kestrel 5500 Weather Meter, Kestrel Meters, Minneapolis, MN). Finally, the canopy cover around each box was measured using a densiometer (Spherical Densiometer Model A; Forest Densiometers, Bartlesville, OK). Canopy cover measurements were taken for each cardinal direction centered on the nest box and mean was calculated. In cases where the nest box abutted a building, the side of the nest box touching the building was considered to have a canopy cover of 100%.

I checked the nest boxes in mid-October when all viable eggs should have hatched (Blouin-Demers et al., 2004; Prior and Weatherhead, 1996) to determine whether or not the nest boxes had been used. I recorded the number of eggs that had hatched, the number of eggs that did not hatch (including both predated and non-viable eggs), and snake species for each nest box.

A preliminary analysis using a repeated measures ANOVA revealed a significant difference in weekly temperatures among the different substrate depths within the nest boxes ($F_{2, 30} = 199$, $p < 0.001$). However, my post-hoc Tukey test found no significant difference in mean weekly temperatures between depths of 20 and 40 cm ($p = 0.196$). Because of this, and because all of the snakes chose to lay their eggs on the bottom of the nest boxes, I excluded the depth of 20 cm measurements from our analyses and used the temperature data at 0 (surface) and 40 (internal) cm in all subsequent analyses.

To investigate the influence of environmental factors on nest box use, I first conducted a principal component analysis combining internal temperature, moisture content, and canopy cover of the nest boxes given my small sample size ($n = 16$). I then used a binomial linear regression to compare nest box use (whether or not they contained eggs) with the first principal component (PC1) scores.

Figure 9. One of the gray ratsnake nest boxes used in this study, based on the designs of the Leeds-Grenville stewardship council (2004).

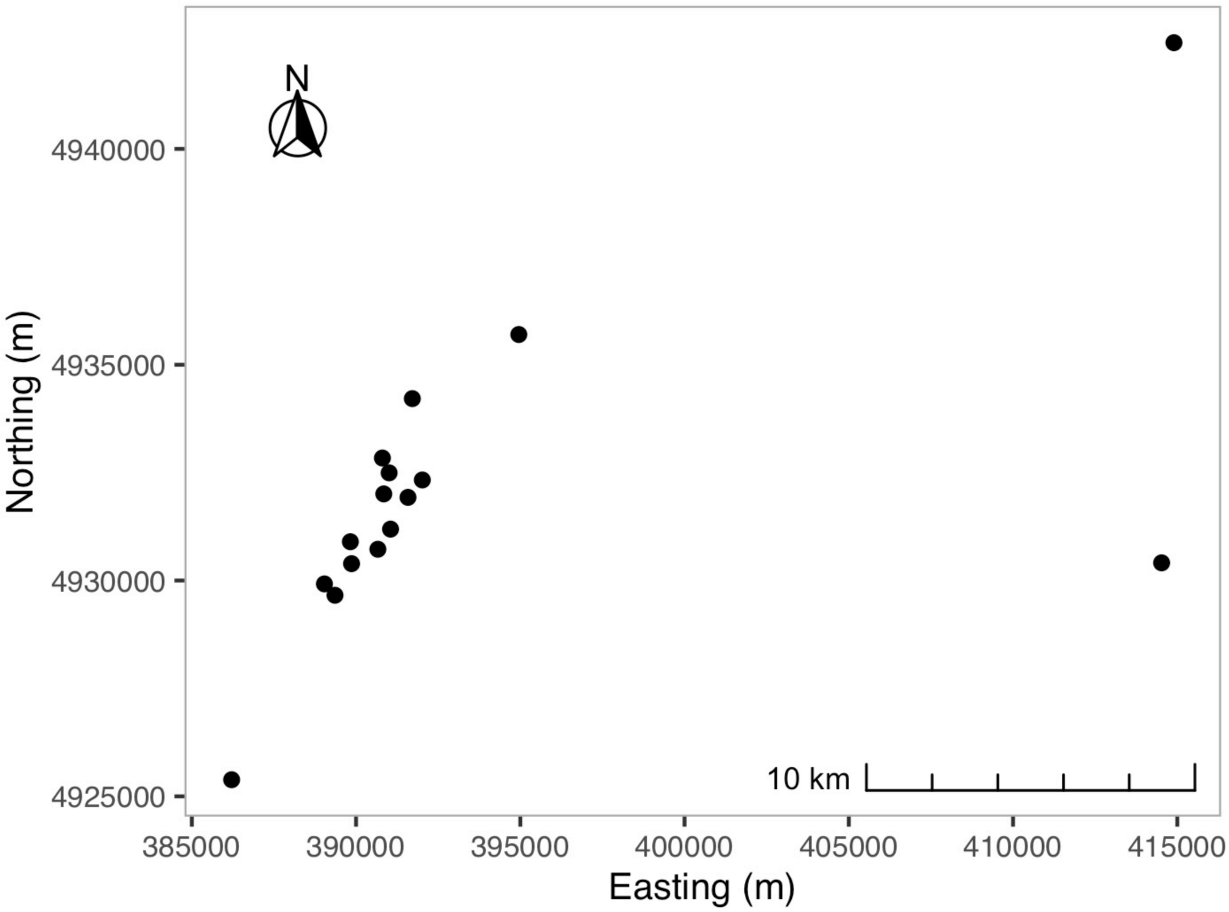

Figure 10. Map of the gray ratsnake nest boxes used in this study. Details such as landscape features, UTM zone, etc. have been omitted due to the sensitivity of this population to persecution and poaching.

3.2 Results

Of the 16 nest boxes, 4 of them contained snake eggs (25% of all nest boxes). The total number of eggs in used nest boxes ranged from 6 to 208 (mean = 106 ± 54 SE); the number of hatched eggs ranged from 5 to 166 (mean = 70 ± 39 SE) and unhatched eggs ranged from 0 to 107 (mean = 35 ± 25 SE). Used nest boxes were slightly warmer than unused nest boxes (Fig. 11). Over the course of the incubation period the average internal temperature of the nest boxes that contained eggs ranged from 29.8 to 41.2°C (mean = 34.6 ± 2.6 SE), and ranged from 25.3 to 33.6°C (mean = 30.3 ± 0.6 SE) for the nest boxes that contained no eggs. The moisture content of nest boxes was also higher than that of unused nest boxes during the first half of incubation (Fig. 12). The average nest box substrate moisture content ranged from 7.4 to 11.6% (mean = 9.4 ± 0.9 SE) for the nest boxes that contained eggs, and 6.1 to 8.4% (mean = 7.4 ± 0.2 SE) for the nest boxes that did not contain any eggs. For the nest boxes that contained eggs canopy cover ranged from 12.3 to 95.4% (mean = 68.3 ± 19.3 SE), and for boxes that did not contain eggs ranged from 2.9 to 96.5% (mean = 65.1 ± 10.0 SE).

PC1 explained 49.1% of the overall variation in environmental variables of the nest boxes (Fig. 13). All environmental variables on PC1 loaded negatively, and loadings were less than -0.5 except for canopy cover (-0.49). Our binomial regression found a near-significant relationship between whether or not a nest box contained eggs and PC1 scores ($z_{15} = -1.796$, $p = 0.072$).

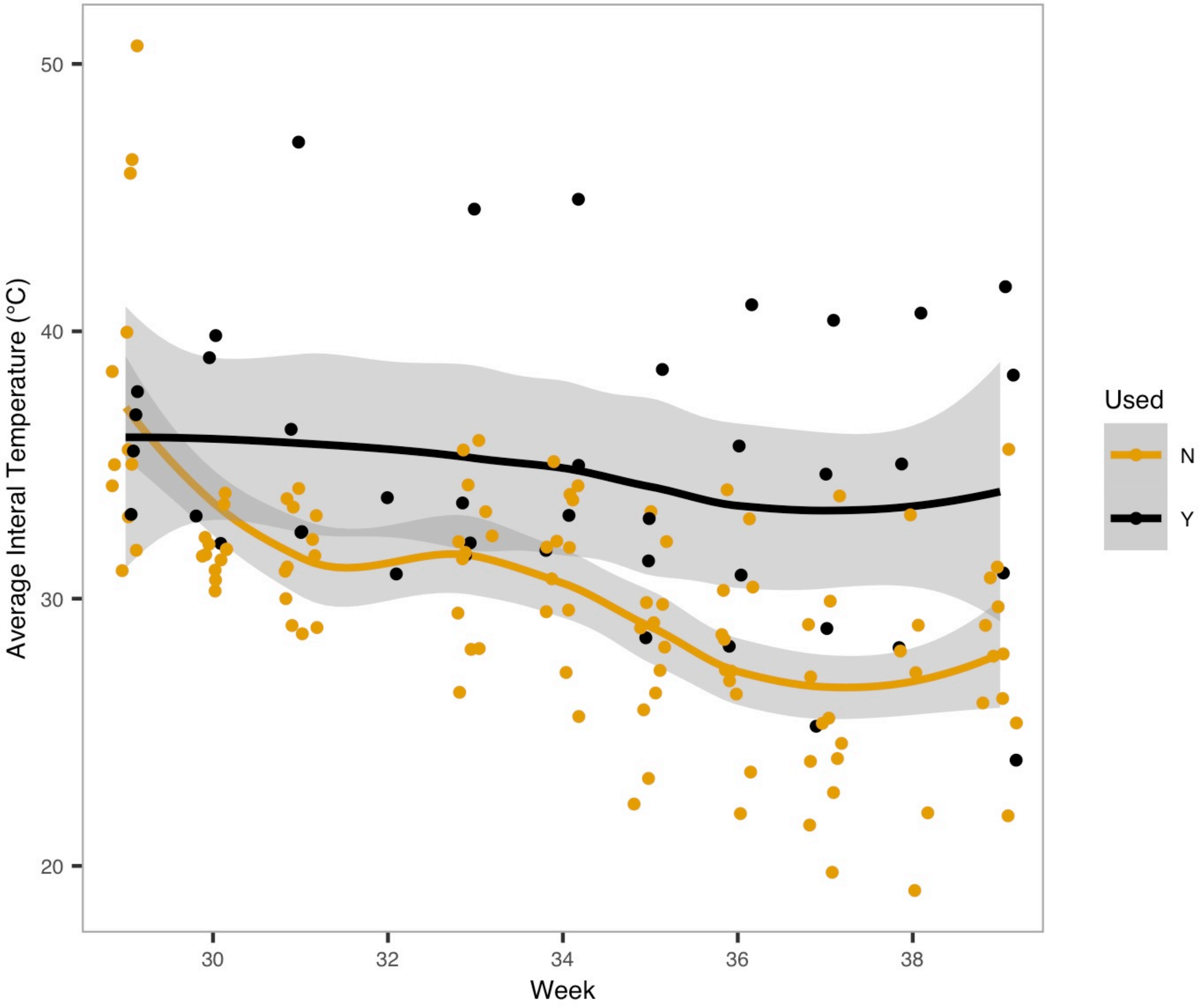

Figure 11. Average internal temperature of used (Y) and unused (N) gray ratsnake nest boxes over the course of the incubation period. The gray bands represent the 95% confidence level for each trend line. The points represent weekly means for each nest box, and have been jittered along the x axis for better visibility.

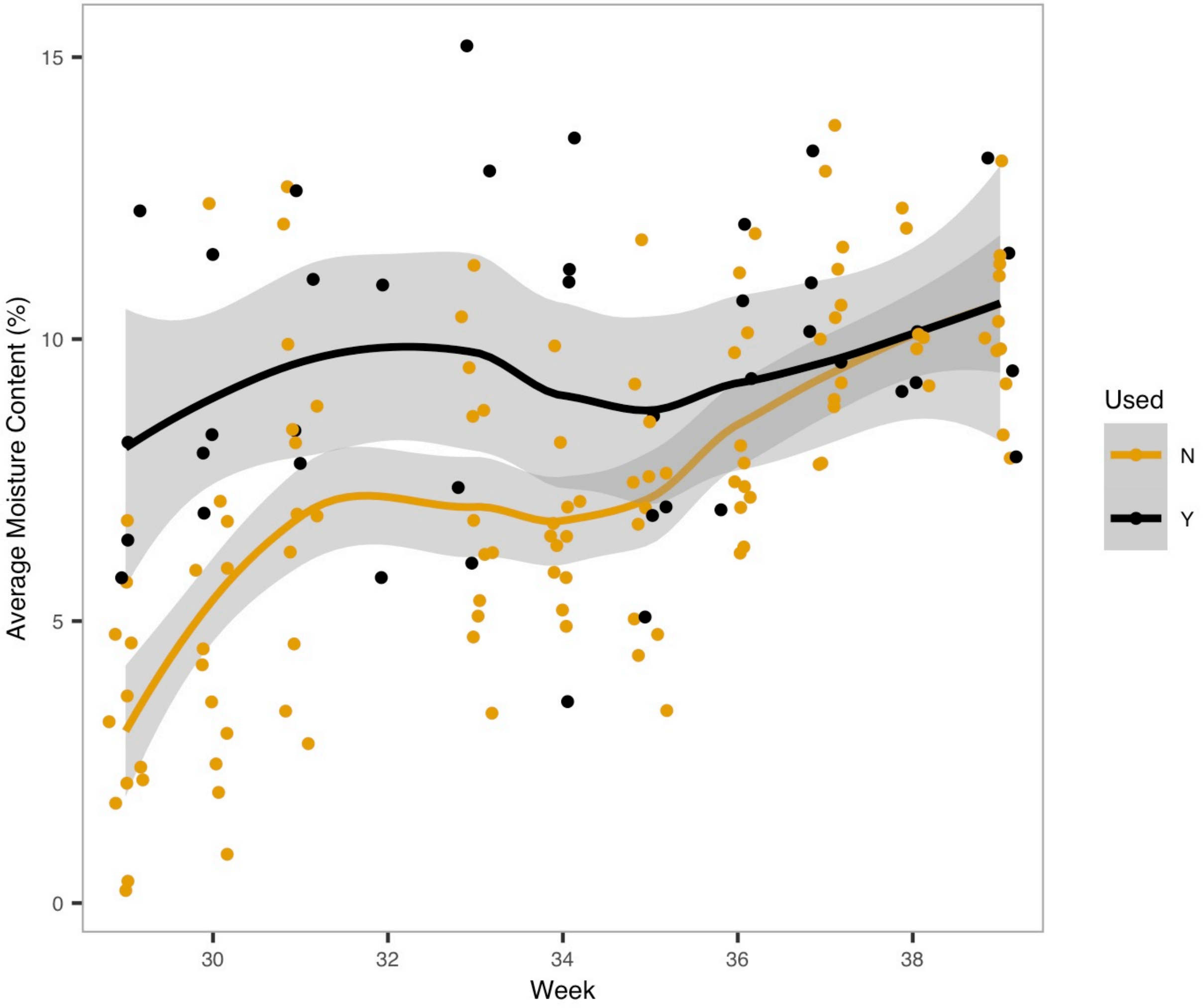

Figure 12. Average moisture content of used (Y) and unused (N) gray ratsnake nest boxes over the course of the incubation period. Other details as in Figure 10.

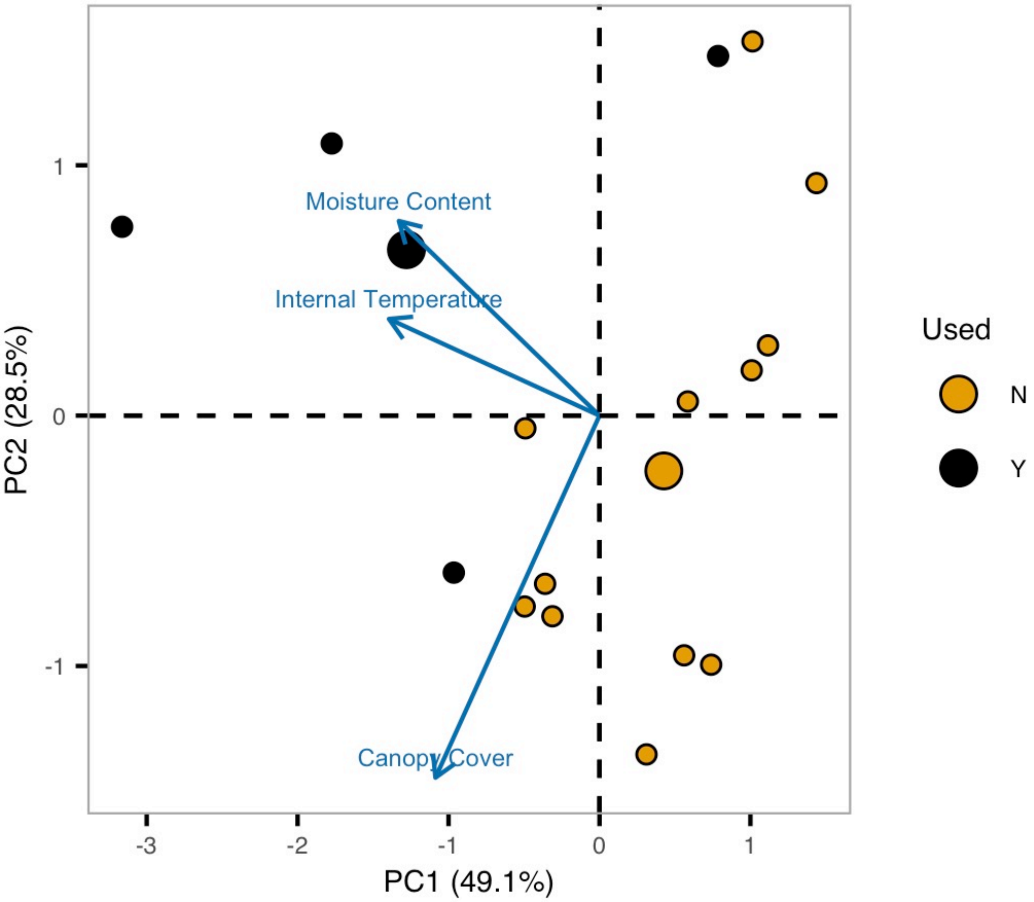

Figure 13. Principal component analysis (PCA) biplot for used (Y) and unused (N) gray ratsnake nest boxes. Environmental variable loadings are represented by the blue arrows. The small points represent the principal component scores for all of the nest boxes, and the large points represent the mean of these scores for each group.

3.2 Discussion

The goal of my study was to identify which environmental variables significantly influence whether or not a gray ratsnake nest box is used, to better inform the placement of these boxes and maximize the likelihood of their use. I found a near significant relationship between the first principal component (a combination of internal temperature, moisture content, and canopy cover) and whether or not a nest box was used. Although the results of this analysis were not significant ($p = 0.072$), preliminary trends in my data suggest that this likely reflects a lack of power given the small sample size of nest boxes that contained snake eggs ($n = 4$). A post-hoc power analysis revealed that power was relatively low at 0.586; to achieve a power = 0.9 for this effect size, I would have required a minimum of 13 used nest boxes in addition to the 12 unused nest boxes. This lack of power is further supported by the similarity of the trends observed in my data to previous studies of snake nesting sites; the nest boxes in my study that were used were on average both warmer and had greater moisture than unused boxes, with less fluctuation in these conditions over the course of the incubation period. Moisture content of used nest boxes also remained relatively consistent throughout the incubation period, with moisture content of unused nest boxes was lower at the start of the incubation period, only reaching similar levels to those of used nest boxes at the end of the incubation period. My data suggest that canopy cover also had an influence on nest box use, although the near orthogonality of vectors within the PCA biplot suggest that canopy cover is directly correlated with either internal temperature or moisture content. This is opposite to previous studies on the decomposition of organic matter, which show a relationship between surrounding canopy cover and both temperature and moisture of decomposing organic matter (Kim, 2000; Shorohova and Kapitsa, 2014).

All of the nest boxes that were used were located within or near to (within 5m) forest edge habitat. This pattern could be because ratsnakes tend to frequent forest edge habitat more than either forest or field habitat, and so are more likely to encounter nest boxes located there (Blouin-Demers & Weatherhead, 2001). However, this association with edge habitat could also be because of the environmental conditions associated with forest edge habitat. Like other decomposing piles of organic matter, the internal temperatures within the nest boxes are determined largely by the productivity of microorganisms within the nesting substrate (Mackensen et al., 2003). Heat is produced as a byproduct of the exothermic reactions needed to break down organic matter; as biological metabolism and chemical reactions increase, so does internal temperature (Zambra et al., 2011). However, the activity of decomposing microorganisms depends on factors such as ambient temperature, moisture, aeration, and substrate quality (Zambra et al., 2011). Further, these relationships are not always linear; the decomposition process is fastest within sites that feature both moderate temperature and moisture intervals (Shorohova and Kapitsa, 2014). Decomposing microorganisms are sensitive to temperatures that are both too low and too high, and to temporal variation in temperature (Shorohova and Kapitsa, 2014). In addition, at low levels of moisture microorganisms such as wood-decaying fungi are slower to grow and colonize a site, whereas at high moisture levels, a reduction in available oxygen makes it difficult for most decomposers to respire properly (Shorohova and Kapitsa, 2014). Although the nest boxes situated in more open habitat such as fields featured ambient temperatures that were warmer due to increased exposure to solar radiation, these nest boxes would be subject to lower relative humidity and greater amounts of wind that increased evaporative loss, which in turn would lead to lower nest box moisture content (Kim, 2000; Yin and Arp, 1993). Alternatively, while nest boxes within forest habitat would be more likely to retain sufficient levels of moisture, the surrounding ambient temperature may have been too low leading to lower internal temperatures of the nest boxes (Mackensen et al., 2003; Shorohova and Kapitsa, 2014; Zambra et al., 2011). Nest boxes within forest edge habitat likely experienced ambient temperatures both high and stable enough to support egg incubation, but are sheltered from an excessive amount of solar radiation and wind that would lead to lower moisture content.

My study also found that ratsnakes prefer to nest within sites with higher temperature profiles than those previously recorded for the study population; in my study the mean temperature of nest boxes that were used ranged from 29.8 to 41.2°C, compared to the range of 22.2 to 31.8 °C described by Blouin-Demers et al. (2004). Further, the nest boxes that remained unused ranged from 25.3 to 33.6°C, well within the range described by Blouin-Demers et al. (2004). Although it is possible that the unused nest boxes were lacking in other aspects (too dry, far from the forest edge, etc.), my study suggests that gray ratsnakes within this population prefer to oviposit within nest sites that are warmer than previously described. This pattern is similar to that observed in female grass snakes (*Natrix natrix*) which oviposit within anthropogenic nest sites featuring significantly greater temperatures than natural nest sites (Löwenborg et al., 2010).

Although I attempted to rigorously investigate the environmental factors that determine the use of gray ratsnake nest boxes, my study had some limitations in addition to the lack of power mentioned earlier. First, while most nest boxes were initially set-up within the same year (2018), my study incorporated four nest boxes that had been installed earlier; one in 2010 (DR01), one in 2014 (KB01), and two in 2017 (EL01, BO01). Of these four older nest boxes, two were used by gray ratsnakes at the time of this study (DR01 and KB01), were known to contain eggs in previous years, and featured the greatest number of eggs. The older age of these nest boxes likely had an influence on their use, as several species of oviparous snakes exhibit high degrees of nest site philopatry (Burger and Zappalorti, 1992). Further, because nesting material from the previous year is incorporated into the new nesting substrate when the boxes are refilled each year, the older boxes likely had a greater and more stable decomposing microorganism community, and thus better substrate quality compared to newer nest boxes. The water holding capacity of wood increases with decay due to an increase in water retention capacity (Pichler et al. 2011). Gravid female snakes may also be more cautious approaching newer habitat features within their environment. Second, although all of the eggs I found within the nest boxes were within the clutch and size range of gray ratsnake eggs, the single clutch found within HU04 overlapped with eastern milksnake (*Lampropeltis triangulum triangulum*) eggs in terms of both clutch and egg size (n = 6, length = 35.8 ± 0.9 mm, width = 17.8 ± 0.8 mm; Rowell, 2012). This box is the used nest box located in the top-right quadrant of the PCA biplot, which appears to be slightly separated from the used nest box grouping (Fig. 13). Although there have been no reports of eastern milksnake eggs found within gray ratsnake nest boxes (LGSC pers. comm.), this species is commonly found within the study area and is known to use similar nest sites to gray ratsnakes such as rotting logs and compost piles (Rowell, 2012). Finally, I failed to record data for other factors known to influence the temperature and moisture within decaying piles of organic matter such as oxygen concentration, soil composition, wind, and tree species within the area (Aponte et al., 2012; Shorohova and Kapitsa, 2014; Zambra et al., 2011).

My study only found a near-significant relationship between a composite principal component variable and nest occupancy, a trend that warrants further investigation. Based on what I have found I make the following provisional recommendations for nest box placement and installation based on the preliminary data of this study and anecdotal evidence: 1) Gray ratsnake nest boxes should be positioned along forest edge habitat, and if possible surrounded by dense, low-growing shrubs in order to decrease moisture loss through wind while still allowing solar radiation to warm the nesting material. 2) The nesting substrate should be wetted thoroughly (damp, but not soaking wet) upon refilling the nest boxes in order to ensure higher levels of substrate moisture content at the start of the incubation period. 3) Time may be needed for the substrate to reach an adequate quality before the nest box will be used; it has been suggested that this usually takes around 3-4 years (depending on location and environmental conditions; LGSC pers. comm.). Future studies should include a larger sample size, collect data over a multi-year period, and include the measurement of other environmental variables such as oxygen concentration, soil composition, wind, and tree species within the area. Given the potential for these nest boxes to support the incubation of such a high number of successful snake egg clutches, they can be an important and relatively simple tool for conserving and monitoring populations of oviparous snake species at risk.

Summary

The goals of my thesis were to evaluate the effectiveness of roadside barrier fencing and artificial nest sites for gray ratsnakes. I found that fencing material, height, and shape, as well as morphological variation within a species, strongly influence the success of roadside exclusion fencing and therefore all need to be considered. Ratsnakes had the least success climbing over fencing that was composed of a more transparent material, fencing that was higher, and fencing with a lip. I also found a significant interaction effect between fencing height and shape; the inclusion of a lip had a greater impact on preventing climbing success for shorter fencing compared to taller fencing. Further, my study also shows that behaviour is an important consideration when designing conservation strategies; ratsnakes attempted to climb fencing that was taller and composed of hardware cloth less often than shorter fencing composed of vinyl sheeting. By exploiting a target species behaviour, more effective and less costly fencing designs can be implemented. In the future, I recommend studies investigate the response of snake species with different morphologies and life histories to different fencing designs. I also found a difference in nest box use by gray ratsnakes, with females preferring to lay their eggs within nest boxes featuring higher internal temperatures and moisture levels, and nest boxes situated on edge habitat. However, due to a lack of power given my sample size, future study on gray ratsnake nest box use is warranted. Overall, my thesis highlights the importance of rigorously studying conservation strategies such as exclusion fencing and artificial nest sites in advance of implementation to achieve higher levels of success and avoid wasting the already limited funding available to conservation projects.

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Appendix 1: Nest Box Data

ID	Eggs	Hatched (%)	Avg. Internal Temp. (° C)	Avg. Weekly Difference in Int. Temp. (° C)	Avg. Moisture Content (%)	Avg. Canopy Cover (%)	Avg. Air Temperature (° C)	Avg. Air Humidity (%)
BO01	0	NA	33.6	5.4	7.2	93	23.3	61.8
DR01	190	87.4	31.6	3.1	11.6	73	23.2	66.9
EL01	0	NA	30.6	10.6	6.7	41	24.4	60.5
HU01	0	NA	31.6	4.6	6.1	96	24.0	64.7
HU02	0	NA	28.9	3.7	7.2	39	24.3	60.0
HU03	0	NA	30.9	5.1	6.6	10	25.9	60.3
HU04	6	100	29.8	4.3	8.5	12	25.2	59.5
HU05	0	NA	27.3	4.7	7.2	86	23.8	63.8
HU06	0	NA	30.8	4.6	7.2	53	24.3	61.8
HU07	0	NA	31.0	6.1	7.8	95	24.5	63.3
HU08	0	NA	25.3	3.6	8.2	93	23.1	65.1
HU09	0	NA	32.0	4.4	8.4	76	23.8	63.1
HU10	0	NA	30.1	4.1	8.3	95	22.5	70.0
HU11	0	NA	31.3	6.2	7.7	3	23.6	63.4
KB01	208	48.6	35.6	4.5	7.4	95	23.2	64.5
QB01	20	25.0	41.4	4.0	10.1	92	24.4	61.7