

Movement and Habitat Selection of Eastern Milksnakes (Lampropeltis triangulum) at Intact and Fragmented Sites

Marcus P. Maddalena¹, Jeffrey R. Row¹, Matthew E. Dyson¹, Gabriel Blouin-Demers², and Bradley C. Fedy¹

Habitat loss and fragmentation are among the greatest threats to wildlife and biodiversity. Reptiles are particularly susceptible to these threats due to high site fidelity, large home ranges, and slow movement rates. To understand behavioral responses of Eastern Milksnakes (*Lampropeltis triangulum*) to fragmentation, we compared home range size and movement rates between a fragmented habitat and an intact habitat. Additionally, we quantified road avoidance and habitat selection in the fragmented habitat. In 2015 and 2016, we collected 453 locations from 17 individuals from Rouge National Urban Park (RNUP), the fragmented study area, using radio-telemetry. We compared our results to a previous study with 1,001 locations from 30 individuals at Queen's University Biological Station (QUBS), our intact study area, collected from 2003 to 2004. We found that home ranges were smaller, but daily movement rate (DMD) and distance-per-move (DPM) were greater in the fragmented study area. We also observed that road crossings by snakes occurred less than expected, suggesting active avoidance of roads. Milksnakes in the fragmented habitat selected locations with a greater number of cover objects within open patches surrounded by high density vegetation, which is consistent with previous findings from the intact habitat. Our findings suggest that Eastern Milksnakes benefit from heterogeneous microhabitats and an abundance of available anthropogenic or natural cover.

UMAN-caused degradation and fragmentation of habitat are among the greatest threats to global biodiversity (Fahrig, 2003; Krauss et al., 2010). The ability of wildlife populations to persist with increasing levels of habitat fragmentation is influenced by species life history and the size and distribution of remnant habitat patches (Wiegand et al., 2005; McKinney, 2006). Patch characteristics vary depending on land use, and along a rural to urban gradient, patches generally become smaller and more isolated, a configuration that favors generalist species and can lead to extirpation of species requiring larger patches (Pickett et al., 2001; McKinney, 2006). The sizes of urban areas are expanding at a rapid rate to accommodate increases in the global human population, 60% of which is projected to live in urban areas (Seto et al., 2012). The majority of this human population growth is expected to take place in areas where existing habitat already faces direct stressors from humans, placing further pressure on wildlife populations as fragmentation increases (McKinney, 2006; Seto et al., 2012).

Roads are one of the most prevalent causes of habitat fragmentation and loss in urban environments (Mader, 1984; Forman and Alexander, 1998) and have been directly linked to an array of impacts on wildlife populations across many taxa (Forman and Alexander, 1998; Fahrig and Rytwinski, 2009; Andrews et al., 2015a; Prokopenko et al., 2017; Lamb et al., 2018). These impacts include increased mortality (Row et al., 2007; Lamb et al., 2018), altered home ranges (Klingenböck et al., 2000), and changes in movement patterns or behavior, such as avoidance (Forman and Alexander, 1998; Shepard et al., 2008; Paterson et al., 2019). In the long term, these factors can lead to changes in population size and demography (e.g., sex ratios), reduced gene flow (Aresco, 2005; Clark et al., 2010), and extirpation or extinction (Germaine and Wakeling, 2001). In urbanizing areas, former rural roads are often widened with a coincident increase in traffic. These changes amplify negative effects as higher traffic intensity and increasing road width are known to further deter vertebrate crossings and increase mortality (Shine et al., 2004; Robson and Blouin-Demers, 2013). As a consequence, species with large home ranges and high site fidelity are unable to access core home range areas, or risk collisions with vehicles (Forman and Alexander, 1998).

Snakes, a relatively slow-moving group of species, may face increased risk when crossing roads (Gregory et al., 1987; Shepard et al., 2008; Andrews et al., 2015b). In many urban areas, the lack of sufficient resources and potential mates in small habitat patches often necessitates road crossings (Ettling et al., 2013, 2016). Increased road-related mortality can have significant long-term effects on snake populations at both the local and landscape levels (Ashley and Robinson, 1996; Row et al., 2007), particularly in northern climates, where individuals have slow growth and long life spans (Gregory and Larsen, 1996; Row et al., 2007; Tuttle and Gregory, 2012, 2014).

In addition to the direct population impacts associated with mortality, roads also impact snake behavior (Gregory et al., 1987; Rouse et al., 2011). Snakes typically take the shortest path possible (Shine et al., 2004) or avoid crossing paved roads altogether (Shepard et al., 2008; Robson and Blouin-Demers, 2013; Paterson et al., 2019). This avoidance can lead to alterations in home range configuration and movement relative to populations not disturbed by development (Rouse et al., 2011; Robson and Blouin-Demers, 2013; Paterson et al., 2019). As a consequence, roads can add to the effects of habitat loss and act as significant barriers to genetic transfer between snake populations, leading to isolation of subpopulations (Row et al., 2012). However, landscapes featuring corridors of moderate quality habitat and several

¹ School of Environment, Resources and Sustainability, University of Waterloo, 200 University Avenue W, Waterloo, Ontario, N2L 3G1 Canada; Email: (BCF) bfedy@uwaterloo.ca. Send reprint requests to BCF.

² Department of Biology, University of Ottawa, 75 Laurier Avenue E, Ottawa, Ontario, K1N 6N5 Canada.

Submitted: 27 January 2019. Accepted: 10 August 2020. Associate Editor: J. D. Litzgus.

^{© 2020} by the American Society of Ichthyologists and Herpetologists 🛟 DOI: 10.1643/CE-19-187 Published online: 14 December 2020

small, suitable habitat patches can allow gene flow in fragmented landscapes (Row et al., 2010, 2012; COSEWIC, 2014).

The Eastern Milksnake (Lampropeltis triangulum, hereafter Milksnake) is a species closely connected with historic human-caused disturbance (COSEWIC, 2014). They are commonly found in rural areas where hibernation and feeding sites, such as buildings and mammal burrows, are abundant, and they also use a variety of open habitats and forest edges (COSEWIC, 2014). The Milksnake is listed as a species of 'Special Concern' at the federal level in Canada, and as a 'specially protected reptile' within the province of Ontario. At the time of our research, the Milksnake was also listed as species of 'Special Concern' in Ontario, but was delisted in 2016 (COSSARO, 2015). These classifications are largely the result of habitat loss, fragmentation, and substantial road mortality. Currently, urban portions of the Milksnake's range lack contemporary records and research (COSEWIC, 2014). Consequently, available information on movement and habitat selection of Milksnakes is derived from relatively intact areas (Row and Blouin-Demers, 2006a), with their responses to fragmented landscapes still poorly understood.

Our primary objective was to quantify the movement and habitat selection of Milksnakes in a fragmented site bordering a major urban center (Toronto, Ontario, Canada). Specifically, we compared movements and habitat selection by individuals in Rouge National Urban Park (herein RNUP) to those of individuals in a more intact natural landscape, Queen's University Biology Station (herein QUBS). We also quantified road crossings to test whether individuals actively avoid roads at RNUP because of the large number of roads surrounding the fragmented site. Quantification of habitat selection at a fragmented urban site and a qualitative comparison to the intact natural site allowed us to develop an understanding of which microhabitat features are important to snakes in urban areas. We predicted avoidance of roads leading to smaller home ranges at the fragmented site. We also expected cover objects to be important to habitat selection and expected the number of these objects to be limited at the fragmented site.

MATERIALS AND METHODS

Study site: Rouge National Urban Park.-Rouge National Urban Park (RNUP) was established in 2015 as a 79 km² reserve located in the Rouge Valley, along the Rouge River and Little Rouge Creek watersheds. The landscape is a mix of agricultural land, natural areas, and cultural heritage sites connecting the Oak Ridges Moraine to Lake Ontario and bordered by heavily urbanized areas to the east and west. The natural areas within Rouge Valley are composed primarily of secondary growth forest interspersed with meadow, along with lowland swamps. Several of these natural areas are restored pastureland and cropland in an early successional state, bordered by hedgerows of mature trees. Cultural heritage sites in Rouge Valley such as stone cottages, foreclosed farmhouses, and barn foundations remain largely intact and can provide refuge to Milksnakes. Rouge Valley is bisected by two major highways, several multi-lane roadways, and two sets of high traffic rail lines. Although all snake locations are not directly within RNUP park boundaries, hereafter we refer to all individuals tracked in and around this site as within the RNUP study site.

Study site: Queen's University Biology Station.—The Queen's University Biology Station (QUBS) study area is a 24 km² reserve located approximately 100 km south of Ottawa, Ontario (Row and Blouin-Demers, 2006a). The study area is characterized by an array of natural secondary growth deciduous forest, rocky outcroppings, and old fields. QUBS has far less fragmentation, with no adjacent development and only one non-major road bisecting the study area (for additional information refer to Row and Blouin-Demers, 2006a; Row et al., 2007).

Snake capture, transmitter implantation, and tracking.—We captured individuals at RNUP during 2015 and 2016 using a large-scale cover board survey or incidentally. We implanted programmable radio-transmitters (produced by Sigma Eight, Aurora, Ontario, Canada) in 17 Milksnakes large enough so that transmitter weight constituted <4% of the snake body mass (Moore and Gillingham, 2006). Transmitter implantation was completed by veterinary professionals at the Toronto Zoo, using a standard intracoelomic method (Webb and Shine, 1997; Lentini et al., 2011). Snakes were anesthetized using isoflurane with a precision vaporizer set up in a Bain circuit. To facilitate induction, we also injected Propofol in the tail vein and used endotracheal intubation using sheaths from intravenous catheters (\sim 14–18 gauge). While the snakes were anesthetized, we ventilated snakes at a volume of 25 mL per kg 2–4 times per minute and monitored their cardiovascular status. Transmitters were fitted for Milksnakes by cutting the transmitter antenna so that it would end 2-3 cm proximal to the vent. The antenna was inserted into a length of silicon tubing that extended 3-4 mm past its end and was sealed with silicone. Transmitters were gas-sterilized prior to implantation. To prevent transmitters from migrating within the body cavity, they were sutured to the body wall and around a rib. Anesthetic was discontinued at the initiation of skin closure, which included sutures and tissue glue to reduce the potential of infecting the incision site. Snakes were injected with meloxicam and enrofloxacin following the procedure and at the end of the holding period prior to release. All implantation methods were permitted by an Ontario Ministry of Natural Resources Wildlife Scientific Collector's Authorization (1080030) and RNUP procedures were approved by the University of Waterloo's Animal Use Committee (15-04).

At QUBS, Milksnakes were captured incidentally or using cover boards in 2003 and 2004 during a study of Black Ratsnake (*Pantherophis spiloides*) hibernacula (Row and Blouin-Demers, 2006a, 2006b; Row et al., 2007). In the QUBS study, 30 Milksnakes were implanted with radio transmitters (produced by Holohil Systems, Carp, Ontario, Canada) constituting <5% of snake body mass following a similar procedure to the one used in the current study (Row and Blouin-Demers, 2006a, 2006b).

At both sites, snakes were provided a 24-hour recovery period in captivity and then released at their capture site. We assumed snake movement was not adversely impacted by transmitters, but acknowledge potential negative effects of transmitters on behavior (Lentini et al., 2011). Following release, we located individuals 2–3 times weekly during the active season (release date to early September), with addi**Table 1.** Number of individual Eastern Milksnakes (*Lampropeltis triangulum*; n = 47) tracked by site and sex, including the mean, minimum, and maximum number of radio-locations (Locations) at Rouge National Urban Park (RNUP) and Queen's University Biology Station (QUBS).

			Locations				
Site	Sex	п	Max	Min	Mean		
RNUP	M	12	39 42	6 18	23.58 31.6		
QUBS	M F	20 10	51 52	9 14	30.75 36.3		

tional observations recorded bi-weekly through October (Row and Blouin-Demers, 2006b). For each observation, we pinpointed the individual and recorded their location using a handheld GPS unit (Garmin International, precision 3–5 m). We recorded 1001 locations of 30 individuals at QUBS across 2003 and 2004, and 453 locations of 17 individuals in RNUP across 2015 and 2016 (Table 1).

Home range size.—We calculated home range size using 95% minimum convex polygons (MCPs) created using the R package 'adehabitat' (Calenge, 2006; Row and Blouin-Demers, 2006b; Moore and Gillingham, 2006; Byer et al., 2017; Sutton et al., 2017). We removed gravid females from home range analysis (Sutton et al., 2017). We calculated home range size for adult males and non-gravid females tracked for a full active season (May to September) at both sites (Moore and Gillingham, 2006; Ettling et al., 2013, 2016; Vanek and Wasko, 2017). For individuals that were not tracked for a full season, we determined whether the entire home range was used by plotting home range size against number of locations per individual (Lawson and Rogers, 1997; Row and Blouin-Demers, 2006a). If home range size reached an asymptote, it was determined that the entire home range was captured and we included the individual in the analysis (Row and Blouin-Demers, 2006a). We excluded five individuals from RNUP due to their reproductive status or non-asymptotic home range sizes, resulting in a total of 12 individuals (8 males, 4 females) for analysis. For the QUBS data, we excluded nine individuals due to reproductive status or non-asymptotic home range, leading to a total of 21 individuals (15 males, 6 females). We used a two-factor ANOVA to assess differences in home range size by site and sex.

Movement distance.—We analyzed movement of all individuals during peak activity season at both sites (May to September; Row et al., 2007; Sutton et al., 2017). For each individual, we calculated daily movement distance (DMD) and distance-per-move (DPM; Gregory et al., 1987). DMD was calculated for each individual as the mean of the estimated distance between two sequential locations divided by the number of days between locations. DPM was calculated as the mean of sequential distances for all locations with movement greater than 5 m from the previous location (Diffendorfer et al., 2005). DPM assesses distances traveled when the individual presumably left refuges. To accomplish this, we eliminated consecutive locations where the individual had not left a refuge and excluded all movements <5 m based on the maximum error of the GPS prior to calculating sequential distances (Diffendorfer et al., 2005). We calculated DMDs for 30 individuals from QUBS (19 males, 11 females) and 17 individuals from RNUP (12 males, 5 females). We examined differences between sex and site for both movement metrics using linear mixed effects models with individual included as a random intercept to control for individual variation in movement (Bolker et al., 2009; Bates et al., 2015).

Road avoidance in a fragmented site.---We quantified road avoidance by individuals at RNUP, our fragmented site. This analysis was not conducted at QUBS, our intact site, because there was only one road in the study area with low traffic rates and few individuals in the proximity of the road. To determine if individuals actively avoided roads, we compared our observed number of road crossings to a randomly generated expected number of road crossings. To accomplish this, we simulated Milksnake movement paths for each individual. Starting from the first location recorded following transmitter implantation, we generated simulated steps by drawing random bearings between 0 and 360° and matched the distance of the observed step (Klingenböck et al., 2000; Row et al., 2007; Robson and Blouin-Demers, 2013). We repeated this process at each consecutive location, resulting in a series of simulated steps that matched observed steps in distance to form a simulated movement path (Klingenböck et al., 2000; Row et al., 2007; Robson and Blouin-Demers, 2013). We compared the mean number of observed road crossings to the mean number of simulated road crossings for each individual snake using a paired t-test (Row et al., 2007). We interpreted fewer observed road crossings relative to simulated crossings as road avoidance.

Habitat selection.—We examined selection of microhabitat components within home ranges at RNUP (i.e., fourth order selection; Johnson, 1980) using fine-scale habitat data of structural variables collected using paired used-available habitat plots. We collected microhabitat data at every other telemetry location per individual. We established random plots by standing at the used location and spinning a compass to select a random bearing, then rolled a 20-sided die, multiplying the outcome by ten to select a random number of steps to walk to an available location (~10–200 m; Row and Blouin-Demers, 2006a). We measured variables at habitat plots when individuals were not present at the locations, which we confirmed with telemetry (~2–14 days after collection of the occurrence record; Table 2).

We developed conditional logistic regression models, effective for comparing paired used and available plots of wildlife (Compton et al., 2002; Row and Blouin-Demers, 2006a; Dyson et al., 2019). We considered a suite of biologically relevant variables known to influence habitat selection of snakes (Table 2). We examined correlations between our candidate variables to detect collinearity and removed any variables that were strongly correlated (r $|\geq 0.60|$). All variables were scaled to a mean of 0, and we generated all combinations of models from our set of biologically relevant variables (Doherty et al., 2012). We ranked candidate models based on Δ AICc values, considering those with Δ AIC < 4 as competing models (Burnham and Anderson, 2002; Arnold, 2010). We qualitatively compared our results to those from QUBS (Row and Blouin-Demers,

Name	Definition					
Vobstruct	Height and density of surrounding vegetation: used a Robel pole to determine the visual minimum and maximum height of vegetation and averaged these values.					
Dedge	Distance to forest edge (>10 clustered trees with adjoining canopy and DBH > 10 cm) to a maximum of 15 m.					
Canopy	Percent canopy cover measured using a densiometer.					
Dcov	Distance to nearest potential cover object (minimum 50 cm x 50 cm), of sufficient size to provide cover for an adult Milksnake.					
Ncov	Number of potential cover objects with 15 m of the location.					
Sumcov	The total area of cover objects available within 15 m of the location. Derived area from length and width measurements of individual objects and totaled their areas.					
VegHt	The average vegetation height within a 1 m radial plot of the exact location. Three measurements were taken randomly and averaged.					
DTree	Distance to the nearest tree having a diameter at breast height >10 cm, and occurring within 15 m of the location.					

Table 2.	Names and	definitions of	f variables used	in modeling	habitat selection	at Eastern	Milksnake	(Lampropelti	is triangulum)	locations in	Rouge
National	Urban Park	(RNUP).									

2006a), as different variables considered between studies precluded a direct quantitative comparison.

We completed all spatial and statistical analysis in R v. 3.6.2 (R Core Team, 2019). For all statistical tests, we used a confidence level of 85% (Cherry, 2008; Johnson, 2008; Arnold, 2010). We report $\bar{x}\pm$ SD unless otherwise noted.

RESULTS

Home range size.—We did not detect a difference in home range size between the sexes ($F_{1,31} = 0.01$, P = 0.92). Milksnakes at RNUP had smaller home ranges (MCP, 7.04±5.82 ha) compared to snakes at QUBS (11.60±8.90 ha; $F_{1,31} = 2.46$, P = 0.13; Fig. 1). We pooled individuals across sex for the plot of between sites comparison.

Movement distance.—We found that DMD at RNUP was on average 22.64 \pm 7.85 m longer than at QUBS ($F_{1,46.46} = 8.32$, P < 0.01) and did not detect a difference between the sexes

 $(F_{1,39.55} = 0.22, P = 0.64)$. We also found that DPM at RNUP was on average 15.62±10.40 m longer than at QUBS ($F_{1,48.92} = 2.26, P = 0.14$) and did not detect a difference between the sexes ($F_{1,47.33} = 0.09, P = 0.76$). Therefore, we pooled movement metrics across sex to plot individual means for visualization. Mean DMD for RNUP was 45.77±35.01 m compared to QUBS where DMD was 29.16±16.12 m (Fig. 1). Mean DPM for RNUP was 56.03±39.04 m compared to individuals at QUBS where DPM was 47.76±21.63 m (Fig. 1).

Road avoidance in a fragmented site.—We did not detect any road crossings in 2015 or 2016 at RNUP despite many locations being in close proximity to roads. For simulated paths, the mean number of crossings per individual was 3.4 ± 0.7 and ranged from 1.3 to 5.1 crossings per path. A paired t-test indicated that the simulated number of crossings per path was greater than the number of observed crossings $(t_{15} = 7.1, P < 0.0001)$.



Fig. 1. Box plots showing differences in home range sizes and movements of Eastern Milksnakes (*Lampropeltis triangulum*) at Rouge National Urban Park (RNUP) and Queen's University Biological Station (QUBS). Home range size was estimated as 95% minimum convex polygons (MCPs), and movements are represented by distance moved per day (DMD; m/day) and distance per move (DPM; m). Gray points overlayed on boxplots represent the raw data. Horizontal lines in boxplots represent the median, outer edges of the boxes represent the 25th and 75th percentile of the data, respectively, whiskers extend from the edge of the box to the maximum and minimum values up to 1.5x the interquartile range, and points plotted beyond whiskers are outliers.

Table 3. All candidate models producing \triangle AICc values < 4 for Eastern Milksnake (*Lampropeltis triangulum*) habitat covariates at the individual location, and potentially contributing to habitat selection at this scale. Refer to Table 2 for definitions and full names of each variable used in modeling.

Model formula	Κ	ΔAICc	Wi
Ncov+Canopy+Dedge+Vobstruct+VegHt	5	0	0.46
Ncov+Canopy+Vobstruct+VegHt	4	2.34	0.14
Ncov+Canopy+Dedge	3	2.66	0.12
Ncov+Canopy+Dedge+VegHt	4	3.26	0.09
Ncov+Canopy+Dedge+Vobstruct	4	3.51	0.08

Habitat selection.—We collected and analyzed data from 236 paired (472 total) presence-absence plots. We found strong correlation between multiple variables related to canopy cover and cover objects. Univariate models ranked based on AICc suggested support for both distance to the nearest tree (Dtree; ΔAICc 0.00) and canopy cover (Canopy; ΔAICc 1.48). We elected to remove Dtree from our candidate set because canopy cover and its associated thermal profile are better predictors of Milksnake occurrence than tree cover (Row and Blouin-Demers, 2006b). Univariate models examining the remaining three variables led to the retention of number of cover objects (Ncov; ΔAICc 0.00) rather than distance to the nearest cover object (Dcov; ΔAICc 4.98) or total area of cover (Sumcov; ΔAICc 35.02).

After reducing our candidate variable set, we tested all combinations of the five remaining variables. Number of cover objects (Ncov) and canopy cover (Canopy) appeared in all five models producing a Δ AIC < 4 (Table 3). In the best performing model, all predictors had 85% confidence intervals that did not overlap zero (Fig. 2). The coefficients from this model indicate Milksnakes select locations with a greater number of cover objects (Ncov), at greater distance to forest edge (Dedge), and with greater visual obstruction (Vobstruct), while they avoid canopy cover (Canopy) and tall vegetation (VegHt; Fig. 2).

DISCUSSION

We described the ecology of Milksnakes in Rouge National Urban Park, a fragmented site surrounded by development, by examining patterns of individual movement rates and habitat selection. Milksnakes had smaller home ranges and made longer movements in the fragmented habitat, but Milksnakes avoided road crossings despite the close proximity of home ranges and hibernacula to roads. At the microhabitat scale, we found selection for heterogeneous habitats with low canopy cover. Consistent with Milksnakes in more intact areas, individuals preferred locations with higher numbers of potential cover objects.

Home ranges were smaller at RNUP and had a large amount of overlap with each other. Though fragmentation can constrain movements (Vignoli et al., 2009), the nonterritorial nature of many snake species allows for overlap in home ranges among individuals, provided sufficient resources are available (Brattstrom, 1974). Increased home range overlap in fragmented sites has been observed in another snake species as a response to constraints on dispersal (Corey and Doody, 2010). The high amount of overlap between home ranges also suggests the search for mates did not require extensive movement at RNUP (Brito, 2003).



Fig. 2. Standardized coefficients and 85% confidence intervals of the top model for habitat selection by Eastern Milksnakes (*Lampropeltis triangulum*) at Rouge National Urban Park (RNUP). Coefficients greater than 0 (horizontal dashed line) indicate selection and coefficients less than 0 indicate avoidance for each respective variable. Definitions and full names of each variable used in modeling can be found in Table 2.

Movement rates in snakes are known to change seasonally (Shew et al., 2012), vary between sexes (Aresco, 2005), and are influenced by the availability of prey and thermal quality of habitat (Brown et al., 1982; King and Duvall, 1990; Madsen and Shine, 1996; Brito, 2003). We suspect our observations of higher movement rates at RNUP are unlikely due to season because movement data were collected during the same time of year at both RNUP and QUBS. However, the temporal gap (11 years) and latitudinal difference between the studies means we cannot completely rule out the potential influence of climatic differences, and snakes at RNUP may have been exposed to warmer temperatures than snakes at QUBS. Past studies examining the influence of anthropogenic features on movement differ regarding whether movement increases or decreases in fragmented areas (Corey and Doody, 2010; Paterson et al., 2019). Snake movement is often constrained by the thermal quality of habitat, with lower movement rates displayed as thermal quality decreases (Harvey and Weatherhead, 2010). QUBS is more forested than RNUP and is known to be a thermally challenging environment as snakes prioritize selection of thermal sites, which could restrict their movement (Row and Blouin-Demers, 2006a). Therefore, it is possible that better thermal quality at RNUP influenced the increased movement we observed, although we did not test thermal quality of the habitats.

We found that Milksnakes avoided road crossings, which is consistent with other snake species in fragmented areas (Miller et al., 2012; Robson and Blouin-Demers, 2013; Siers et al., 2014; Paterson et al., 2019). Despite Milksnake locations in close proximity to a number of roads, we did not detect any road crossings, which might suggest that areas in proximity to roads provide adequate habitat, including open 852

canopy for basking, foraging, or preferred nesting substrate (COSEWIC, 2014; Paterson et al., 2019). Larger snake species are less likely to avoid crossing roads (Row et al., 2007) because their larger home ranges necessitate crossings in urban areas (Bonnet et al., 1999). Milksnakes are mediumsized snakes and individuals may not have been required to cross roads to satisfy life cycle requirements, such as locating foraging habitats, hibernacula, or mates. Our observations of no road crossings, small home range size, and longer movements suggest that RNUP likely provides adequate resources for Milksnakes. In areas with less habitat, we might predict more frequent road crossings to satisfy life cycle requirements and subsequently increased mortality risk. Therefore, studies that investigate techniques to mitigate road mortality (Ashley and Robinson, 1996; Ashley et al., 2007) or facilitate crossing in fragmented habitats (Colley et al., 2017; Markle et al., 2017) may prove beneficial to the conservation of Milksnakes and other snake species at risk.

Our analysis of individual habitat selection revealed that fine scale habitat structure and heterogeneity of successional habitat is important for Milksnakes. Individuals selected greater overall vegetation density and lower vegetation height, consistent with a trade-off between thermoregulatory benefit and predation risk. Avian species are significant predators of snakes (Webb and Whiting, 2005) and the increased vegetation density likely provides some visual obstruction, while lower vegetation height results in greater radiant heat. Given that open habitats have greater thermal quality for Milksnakes (Row and Blouin-Demers, 2006b), snakes are likely selecting locations with greater sun exposure for basking (Charland and Gregory, 1995). In addition, Milksnakes in RNUP preferred locations with a higher abundance of cover objects, which is consistent with the QUBS study (Row and Blouin-Demers, 2006b). However, the type of cover objects varied between the studies with primarily rock cover objects at QUBS (Row and Blouin-Demers, 2006a) being replaced with building foundations and anthropogenic debris (e.g., tin sheet) at RNUP. Therefore, Milksnakes appear to be adaptable to urban environments to satisfy habitat requirements.

Our results demonstrate the benefit of conservation reserves in urban areas, such as RNUP, to reptile conservation. While we observed differences in home range and movement patterns at our fragmented site compared to a more intact site, snakes appeared to still be able to satisfy habitat requirements without crossing roads. These factors combined indicate that individuals at RNUP are not as adversely affected by roads as a direct mortality source as we had hypothesized and that RNUP, though fragmented, may have suitably large habitat patches remaining to allow persistence of Milksnakes. Therefore, to facilitate conservation of Milksnakes in fragmented habitats, we recommend that managers ensure that adequate vegetative cover and cover objects are present, including human-made cover objects (e.g., tin sheeting, rock piles, old buildings). In addition, for habitat patches smaller than RNUP, we suggest considerations be taken to promote road crossings, such as underpasses (Colley et al., 2017; Markle et al., 2017), to ensure Milksnakes can satisfy all necessary life-history requirements. Further research that investigates survival and structural and functional connectivity of populations in fragmented habitats would greatly improve our knowledge and benefit conservation efforts for Milksnakes and other at-risk snake species.

ACKNOWLEDGMENTS

We thank the Toronto Zoo, specifically Andrew Lentini and Paul Yanuzzi, and Leonardo Cabrera at Parks Canada for their assistance and support in undertaking this project. We also thank Sarantia Katsaras, Catherine Falardeau Marcoux, and our many volunteers for assistance in data collection. Funding for this project was provided by the Species at Risk Research Fund for Ontario and by Parks Canada. We also acknowledge the support of the Canadian Foundation for Innovation and the Natural Sciences and Engineering Research Council of Canada (NSERC) to BCF (funding reference number 50503-10694).

LITERATURE CITED

- Andrews, K. M., T. A. Langen, and R. P. J. H. Struijk. 2015a. Reptiles: overlooked but often at risk from roads, p. 271– 280. *In*: Handbook of Road Ecology. R. van der Ree, D. J. Smith, and C. Grilo (eds.). John Wiley & Sons, Ltd, Oxford, UK.
- Andrews, K. M., P. Nanjappa, and P. D. Riley. 2015b. Roads and Ecological Infrastructure: Concepts and Applications for Small Animals. Johns Hopkins University Press, Baltimore, Maryland.
- **Aresco, M. J.** 2005. The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biological Conservation 123:37–44.
- Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's information criterion. Journal of Wildlife Management 74:1175–1178.
- Ashley, E. P., A. Kosloski, and S. A. Petrie. 2007. Incidence of intentional vehicle–reptile collisions. Human Dimensions of Wildlife 12:137–143.
- Ashley, E. P., and J. T. Robinson. 1996. Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Canadian Field Naturalist 110:403–412.
- **Bates, D., M. Mächler, B. Bolker, and S. Walker.** 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution 24:127–135.
- Bonnet, X., G. Naulleau, and R. Shine. 1999. The dangers of leaving home: dispersal and mortality in snakes. Biological Conservation 89:39–50.
- **Brattstrom**, **B. H.** 1974. The evolution of reptilian social behavior. American Zoologist 14:35–49.
- **Brito**, J. C. 2003. Seasonal variation in movements, home range, and habitat use by male *Vipera latastei* in northern Portugal. Journal of Herpetology 37:155–160.
- Brown, W. S., D. W. Pyle, K. R. Greene, and J. B. Friedlaender. 1982. Movements and temperature relationships of timber rattlesnakes (*Crotalus horridus*) in northeastern New York. Journal of Herpetology 16:151.
- Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Second edition. Springer, New York.
- Byer, N. W., S. A. Smith, and R. A. Seigel. 2017. Effects of site, year, and estimator choice on home ranges of bog

turtles (*Glyptemys muhlenbergii*) in Maryland. Journal of Herpetology 51:68–72.

- **Calenge**, C. 2006. The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. Ecological Modelling 197:516–519.
- Charland, M. B., and P. T. Gregory. 1995. Movements and habitat use in gravid and nongravid female garter snakes (Colubridae: *Thamnophis*). Journal of Zoology 236:543–561.
- Cherry, S. 2008. Statistical tests in publications of The Wildlife Society. Wildlife Society Bulletin 26:947–953.
- Clark, R. W., W. S. Brown, R. Stechert, and K. R. Zamudio. 2010. Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. Conservation Biology 24:1059–1069.
- Colley, M., S. C. Lougheed, K. Otterbein, and J. D. Litzgus. 2017. Mitigation reduces road mortality of a threatened rattlesnake. Wildlife Research 44:48–59.
- Compton, B. W., J. M. Rhymer, and M. McCollough. 2002. Habitat selection by wood turtles (*Clemmys insculpta*): an application of paired logistic regression. Ecology 83:833– 843.
- Corey, B., and J. S. Doody. 2010. Anthropogenic influences on the spatial ecology of a semi-arid python. Journal of Zoology 281:293–302.
- **COSEWIC**. 2014. COSEWIC assessment and status report on the Eastern Milksnake *Lampropeltis triangulum* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa, ON. https://wildlife-species.canada.ca/ species-risk-registry/virtual_sara/files/cosewic/sr_ Eastern%20Milksnake_2014_e.pdf
- COSSARO. 2015. Ontario species at risk evaluation report for eastern milksnake (*Lampropeltis triangulam*). Committee on the Status of Species at Risk in Ontario. Ottawa, ON. http:// cossaroagency.ca/wp-content/uploads/2017/06/ Accessible_COSSARO-evaluation-Eastern-milksnake.pdf
- Diffendorfer, J. E., C. Rochester, R. N. Fisher, and T. K. Brown. 2005. Movement and space use by coastal rosy boas (*Lichanura trivirgata roseofusca*) in coastal southern California. Journal of Herpetology 39:24–36.
- Doherty, P. F., G. C. White, and K. P. Burnham. 2012. Comparison of model building and selection strategies. Journal of Ornithology 152:317–323.
- **Dyson, M. E., S. M. Slattery, and B. C. Fedy.** 2019. Microhabitat nest-site selection by ducks in the boreal forest. Journal of Field Ornithology 90:348–360.
- Ettling, J. A., L. A. Aghasyan, A. L. Aghasyan, and P. G. Parker. 2013. Spatial ecology of Armenian vipers, *Montivipera raddei*, in a human-modified landscape. Copeia 2013:64–71.
- Ettling, J. A., L. A. Aghasyan, A. L. Aghasyan, and P. G. Parker. 2016. Spatial ecology of Armenian vipers, *Montivipera raddei*, in two different landscapes: human-modified vs. recovered-natural. Russian Journal of Herpetology 23:93–102.
- Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution and Systematics 34:487–515.
- Fahrig, L., and T. Rytwinski. 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecology and Society 14:1–20.
- Forman, R. T. T., and L. E. Alexander. 1998. Roads and their major ecological effects. Annual Review of Ecology and Systematics 29:207–231.

- Germaine, S. S., and B. F. Wakeling. 2001. Lizard species distributions and habitat occupation along an urban gradient in Tucson, Arizona, USA. Biological Conservation 97:229–237.
- Gregory, P. T., and K. W. Larsen. 1996. Are there any meaningful correlates of geographic life-history variation in the garter snake, *Thamnophis sirtalis*? Copeia 1996:183–189.
- Gregory, P. T., J. M. MacCartney, and K. W. Larsen. 1987. Spatial patterns and movements, p. 366–395. *In*: Snakes: Ecology and Evolutionary Biology. R. A. Seigel, J. T. Collins, and S. S. Novak (eds.). Macmillan, New York.
- Harvey, D. S., and P. J. Weatherhead. 2010. Habitat selection as the mechanism for thermoregulation in a northern population of Massasauga rattlesnakes (*Sistrurus catenatus*). Écoscience 17:411–419.
- **Johnson**, **D. H.** 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61:65–71.
- **Johnson**, **D. H.** 2008. The insignificance of statistical significance testing. Journal of Wildlife Management 63: 763–772.
- King, M. B., and D. Duvall. 1990. Prairie rattlesnake seasonal migrations: episodes of movement, vernal foraging and sex differences. Animal Behaviour 39:924–935.
- Klingenböck, A., K. Osterwalder, and R. Shine. 2000. Habitat use and thermal biology of the "land mullet" *Egernia major*, a large scincid lizard from remnant rain forest in southeastern Australia. Copeia 2000:931–939.
- Krauss, J., R. Bommarco, M. Guardiola, R. K. Heikkinen, A. Helm, M. Kuussaari, R. Lindborg, E. Öckinger, M. Pärtel, J. Pino, J. Pöyry, K. M. Raatikainen, A. Sang, C. Stefanescu . . . I. Steffan-Dewenter. 2010. Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. Ecology Letters 13: 597–605.
- Lamb, C. T., G. Mowat, A. Reid, L. Smit, M. Proctor, B. N. McLellan, S. E. Nielsen, and S. Boutin. 2018. Effects of habitat quality and access management on the density of a recovering grizzly bear population. Journal of Applied Ecology 55:1406–1417.
- Lawson, E. J., and A. R. Rogers. 1997. Differences in homerange size computed in commonly used software programs. Wildlife Society Bulletin 25:721–729.
- Lentini, A. M., G. J. Crawshaw, L. E. Licht, and D. J. McLelland. 2011. Pathologic and hematologic responses to surgically implanted transmitters in eastern Massasauga rattlesnakes (*Sistrurus catenatus catenatus*). Journal of Wildlife Diseases 47:107–125.
- Mader, H.-J. 1984. Animal habitat isolation by roads and agricultural fields. Biological Conservation 29:81–96.
- Madsen, T., and R. Shine. 1996. Seasonal migration of predators and prey—a study of pythons and rats in Tropical Australia. Ecology 77:149–156.
- Markle, C. E., S. D. Gillingwater, R. Levick, and P. Chow-Fraser. 2017. The true cost of partial fencing: evaluating strategies to reduce reptile road mortality. Wildlife Society Bulletin 41:342–350.
- McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation 127:247–260.
- Miller, G. J., L. L. Smith, S. A. Johnson, and R. Franz. 2012. Home range size and habitat selection in the Florida pine

snake (*Pituophis melanoleucus mugitus*). Copeia 2012:706–713.

- Moore, J. A., and J. C. Gillingham. 2006. Spatial ecology and multi-scale habitat selection by a threatened rattlesnake: the Eastern Massasauga (*Sistrurus catenatus catenatus*). Copeia 2006:742–751.
- Paterson, J. E., J. Baxter-Gilbert, F. Beaudry, S. Carstairs, P. Chow-Fraser, C. B. Edge, A. M. Lentini, J. D. Litzgus, C. E. Markle, K. McKeown, J. A. Moore, J. M. Refsnider, J. L. Riley, J. D. Rouse . . . C. M. Davy. 2019. Road avoidance and its energetic consequences for reptiles. Ecology and Evolution 9:9794–9803.
- Pickett, S. T. A., M. L. Cadenasso, J. M. Grove, C. H. Nilon, R. V. Pouyat, W. C. Zipperer, and R. Costanza. 2001. Urban ecological systems: linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. Annual Review of Ecology and Systematics 32:127– 157.
- **Prokopenko, C. M., M. S. Boyce, and T. Avgar.** 2017. Characterizing wildlife behavioural responses to roads using integrated step selection analysis. Journal of Applied Ecology 54:470–479.
- **R** Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- **Robson**, L. E., and G. Blouin-Demers. 2013. Eastern hognose snakes (*Heterodon platirhinos*) avoid crossing paved roads, but not unpaved roads. Copeia 2013:507–511.
- Rouse, J. D., R. J. Willson, R. Black, and R. J. Brooks. 2011. Movement and spatial dispersion of *Sistrurus catenatus* and *Heterodon platirhinos*: implications for interactions with roads. Copeia 2011:443–456.
- **Row, J. R., and G. Blouin-Demers.** 2006a. Thermal quality influences habitat selection at multiple spatial scales in milksnakes. Ecoscience 13:443–450.
- **Row, J. R., and G. Blouin-Demers.** 2006b. Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. Oecologia 148:1–11.
- Row, J. R., G. Blouin-Demers, and S. C. Lougheed. 2010. Habitat distribution influences dispersal and fine-scale genetic population structure of eastern foxsnakes (*Mintonius gloydi*) across a fragmented landscape. Molecular Ecology 19:5157–5171.
- **Row, J. R., G. Blouin-Demers, and S. C. Lougheed.** 2012. Movements and habitat use of eastern foxsnakes (*Pantherophis gloydi*) in two areas varying in size and fragmentation. Journal of Herpetology 46:94–99.
- Row, J. R., G. Blouin-Demers, and P. J. Weatherhead. 2007. Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). Biological Conservation 137:117–124.

- Seto, K. C., B. Guneralp, and L. R. Hutyra. 2012. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences of the United States of America 109: 16083–16088.
- Shepard, D. B., A. R. Kuhns, M. J. Dreslik, and C. A. Phillips. 2008. Roads as barriers to animal movement in fragmented landscapes. Animal Conservation 11:288–296.
- Shew, J. J., B. D. Greene, and F. E. Durbian. 2012. Spatial ecology and habitat use of the western foxsnake (*Pantherophis vulpinus*) on Squaw Creek National Wildlife Refuge (Missouri). Journal of Herpetology 46:539–548.
- Shine, R., M. Lemaster, M. Wall, T. Langkilde, and R. Mason. 2004. Why did the snake cross the road? Effects of roads on movement and location of mates by garter snakes (*Thamnophis sirtalis parietalis*). Ecology and Society 9:1–9.
- Siers, S. R., J. A. Savidge, and R. N. Reed. 2014. Invasive brown treesnake movements at road edges indicate roadcrossing avoidance. Journal of Herpetology 48:500–505.
- Sutton, W. B., Y. Wang, C. J. Schweitzer, and C. J. W. McClure. 2017. Spatial ecology and multi-scale habitat selection of the copperhead (*Agkistrodon contortrix*) in a managed forest landscape. Forest Ecology and Management 391:469–481.
- **Tuttle, K. N., and P. T. Gregory.** 2012. Growth and maturity of a terrestrial ectotherm near its northern distributional limit: does latitude matter? Canadian Journal of Zoology 90:758–765.
- Tuttle, K. N., and P. T. Gregory. 2014. Reproduction of the Plains garter snake, *Thamnophis radix*, near its northern range limit: more evidence for a "fast" life history. Copeia 2014:130–135.
- Vanek, J. P., and D. K. Wasko. 2017. Spatial ecology of the eastern hog-nosed snake (*Heterodon platirhinos*) at the northeastern limit of its range. Herpetological Conservation and Biology 12:109–118.
- Vignoli, L., I. Mocaer, L. Luiselli, and M. A. Bologna. 2009. Can a large metropolis sustain complex herpetofauna communities? An analysis of the suitability of green space fragments in Rome. Animal Conservation 12:456–466.
- Webb, J. K., and R. Shine. 1997. Out on a limb: conservation implications of tree-hollow use by a threatened snake species (*Hoplocephalus bungaroides*: Serpentes, Elapidae). Biological Conservation 81:21–33.
- Webb, J. K., and M. J. Whiting. 2005. Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. Oikos 110:515–522.
- Wiegand, T., E. Revilla, and K. A. Moloney. 2005. Effects of habitat loss and fragmentation on population dynamics. Conservation Biology 19:108–121.