# Thermal quality influences habitat selection at multiple spatial scales in milksnakes[1]

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*Abstract*: Factors influencing habitat selection may be scale dependent, leading to different selection patterns at different spatial scales. By limiting habitat-selection studies to a single scale, important selection patterns could be missed. Despite this danger, many studies investigate habitat selection at a single scale, often ignoring macro-habitat selection: the selection of a home range within the study area. We investigated macro- and micro-habitat selection in milksnakes. Because of the importance of thermoregulation to ectotherms, we predicted that snakes would select habitats of high thermal quality at both micro- and macro-habitat scales. In 2003–2004, we located 25 individuals 890 times and characterized the habitat in detail at 279 locations used by milksnakes and at 279 paired random locations. Open habitats (fields, rocky outcrops, marshes) and edges have environmental temperatures that deviate less from the preferred body temperature range of milksnakes and offer characteristics that facilitate thermoregulation compared to forest. At the macro- and micro-habitat scales, milksnakes preferred habitats of high thermal quality: they used fields and rocky outcrops more than forests. Milksnakes also preferred edges at both scales. In addition, milksnakes preferred locations with open canopy and many rocks at the micro-habitat scale. These results support the notion that thermal quality influences habitat use in ectotherms and strengthen the idea that habitat-use studies should be conducted at more than one spatial scale to gain a complete understanding of the factors affecting selection.
*Keywords*: *Lampropeltis triangulum*, macro-habitat, micro-habitat, Ontario, paired logistic regression, remote sensing.

*Résumé* : Les facteurs influençant la sélection d'habitat peuvent être tributaires de l'échelle, ce qui peut conduire à des patrons de sélection différents à différentes échelles spatiales. En limitant les études de sélection d'habitat à une seule échelle, il est possible de manquer certains patrons de sélection importants. Malgré ce risque, plusieurs études de sélection d'habitat ignorent la sélection au niveau du macro-habitat, *i.e.* la sélection du domaine vital dans l'aire d'étude. Nous avons étudié la sélection du macro et du micro-habitat chez la couleuvre tachetée. Puisque la thermorégulation est un facteur très important chez les ectothermes, nous avons prédit que les couleuvres devraient sélectionner des habitats de haute qualité thermale autant au niveau du micro que du macro-habitat. En 2003-2004, nous avons localisé 25 individus 890 fois et caractérisé leur habitat en détail pour 279 sites de localisation de couleuvres et 279 autres sites appariés choisis au hasard. Les habitats ouverts (champs, affleurements rocheux, marais) et bordures ont des températures environnementales qui s'écartent moins des températures corporelles préférées des couleuvres et offrent des caractéristiques qui facilitent la thermorégulation en comparaison avec les habitats forestiers. À l'échelle du macro et du micro-habitat, les couleuvres ont préféré les habitats de haute qualité thermale : elles ont utilisé les champs et les affleurement rocheux plus que la forêt. Les couleuvres ont aussi préféré les bordures aux deux échelles spatiales. De plus, à l'échelle du micro-habitat, les couleuvres ont préféré les sites ouverts et rocheux. Ces résultats supportent la notion que la qualité thermale de l'habitat influence l'utilisation par les ectothermes et renforcent l'idée que les études d'utilisation d'habitat devraient être menées à plus d'une échelle spatiale afin d'obtenir une compréhension complète des facteurs influençant la sélection.
*Mots-clés* : *Lampropeltis triangulum*, macro-habitat, micro-habitat, Ontario, régression logistique appariée, télédétection.

*Nomenclature*: Conant & Collins, 1991.

## Introduction

Habitat use refers to the preference an individual has for a particular habitat, while studies of habitat selection seek to determine the factors that influence the individual to use that same habitat (Wiens, 1976; Reinert, 1993). An organism has many choices when selecting a habitat, and most of these choices can occur at multiple spatial scales (Orians & Wittenberger, 1991). Different selection pressures and limiting factors may be present that can lead to distinct patterns of habitat use at various spatial scales (Rettie & Messier, 2000; Luck, 2002; Stephens *et al.,* 2003). Limiting

the study of habitat use to a single spatial scale can cause some or all of these patterns to be missed.

Macro- and micro-habitat use have often been equated with the selection of a home range and selection of locations within that home range (Johnson, 1980), respectively, and have been used in describing the ecology of numerous species (Blouin-Demers & Weatherhead, 2001a; Luck, 2002; Boyce *et al.,* 2003). Although there have been many studies on micro-habitat use, few studies have investigated macro-habitat use or selection at multiple spatial scales. Habitat-use studies on birds and mammals are sometimes conducted at more than one spatial scale, but, surprisingly, this is rarely the case for reptiles. Compton, Rhymer, and McCollough (2002) examined habitat use in wood turtles at two spatial scales and found selection at both scales. At

the watershed level, wood turtles used activity areas that were partially forested, but within their activity areas turtles used locations with low canopy cover. This was believed to represent a trade-off between feeding and thermoregulation, and it highlights the importance of scale when evaluating habitat use in reptiles.

Reptiles have been shown to select habitats based on the distribution of prey (Madsen & Shine, 1996) and hibernation sites (Reinert & Kodrich, 1982; Reinert, 1993), based on predator avoidance (Webb & Whiting, 2005), and based on thermoregulatory requirements (Reinert, 1984; Blouin-Demers & Weatherhead, 2001a). Because of the importance of thermoregulation to fitness in ectotherms (Christian & Tracy, 1981; Huey & Kingsolver, 1989), however, temperature is believed to be one of the most important factors influencing habitat selection in terrestrial squamates (Reinert, 1993). Thermoregulation is particularly important to reptiles in climatic extremes (northern climates and hot deserts) because environmental temperatures ($T_e$) are far from optimal temperatures and the lack of thermoregulation results in body temperatures ($T_b$) that drastically decrease performance (Blouin-Demers & Weatherhead, 2001b).

Row and Blouin-Demers (2006) measured the preferred body temperature range ($T_{set}$) of milksnakes and $T_e$ available in different habitats and seasons. Hertz, Huey, and Stevenson (1993) defined thermal quality of the habitat as the magnitude of the difference between $T_e$ and $T_{set}$, and, based on this definition, Row and Blouin-Demers (2006) determined that thermal quality was generally low in our study area, because $T_e$ were far from $T_{set}$ on average. Thermal quality was higher in open habitats than in forest in all seasons, however, and milksnakes are able to maintain $T_b$ within $T_{set}$ for most of the day in open habitats by shuttling between sun and cover. Edges offer the thermal characteristics of both open habitats and forest, and edges have been shown to be important for thermoregulation in snakes (Blouin-Demers & Weatherhead, 2001b).

We conducted this study on eastern milksnakes at the northern extreme of their range in a temperate climate. Because of the low thermal quality of this environment, we expected thermoregulation to be particularly important for milksnakes, and thus we predicted that thermal quality would be an important factor affecting habitat use. Specifically, we predicted that milksnakes would prefer open habitats and edges at both macro- and micro-habitat scales because these habitats have the highest thermal quality.

## Methods

### Study area and study species

We conducted this study at the Queen's University Biology Station, 100 km south of Ottawa, Ontario (44° 34' N, 76° 19' W) from August 2002 to November 2004. The study area was approximately 8 × 3 km and was characterized by rolling terrain covered with second-growth deciduous forest interspersed with numerous rocky outcrops, small lakes, old fields, and marshes. Eastern milksnakes (*Lampropeltis triangulum*) are medium-sized (up to 92 cm snout–vent length and 210 g) colubrids that feed mostly on small mammals. Density is low, but milksnakes occur throughout the study area.

### Radio-telemetry

We captured milksnakes at black ratsnake communal hibernacula monitored during spring emergence (Blouin-Demers *et al.,* 2000); others were captured opportunistically throughout the active season. For each snake, we probed the base of the tail for the presence of hemipenes to determine sex, measured snout–vent length (SVL) with a flexible measuring tape (Blouin-Demers, 2003), determined mass with an electronic scale, and marked the individual by injecting a passive integrated transponder (PIT tag) subcutaneously.

We implanted radio-transmitters in a subset of individuals that were large enough (at least 130 g) to bear the unit (Model SB-2T, battery life of 12 months at 20 °C, Holohil Systems Inc., Carp, Ontario, Canada). The transmitter weighed 5.5 g and never exceeded 5% of the snake's mass. We intended to implant radio-transmitters in an equal number of females and males, but this was not possible because we captured fewer females than males and also because females are slightly smaller than males, therefore, fewer females were large enough to bear the transmitter. Implantations were performed under sterile conditions and isoflurane anaesthesia (Blouin-Demers *et al.,* 2000; Weatherhead & Blouin-Demers, 2004).

We released the snakes at their point of capture 1 d after implantation and located them every 2–3 d with a telemetry receiver (Wildlife Materials, Murphysboro, Illinois, USA) and a directional Yagi antenna. At each location, we recorded the UTM coordinates (NAD83 datum) with a GPSmap76 (Garmin, Olathe, Kansas, USA), the position of the snake and its behaviour.

### Home ranges

Minimum convex polygon (MCP) is the most commonly used home range estimator. MCP is based on the smallest possible convex polygon that encompasses all known locations of a given animal. It is widely used because it is very simple to conceptualize and it does not rely on the data having an underlying statistical distribution (Powell, 2000). Despite its wide use and simplicity, however, MCP only provides a crude outline of an animal's home range and often includes large areas never used by the animal. Also, MCP ignores patterns of selection within the home range (Powell, 2000; Taulman & Seaman, 2000).

To examine selection of habitat within a home range, estimators must be used to determine the intensity of use. Kernel home range estimators have been shown to be the most consistent and accurate of the estimators available (Worton, 1989; Seaman & Powell, 1996). Kernel estimators are non-parametric and produce a distribution that estimates the likelihood of finding the animal at any particular location within its home range (Powell, 2000). One drawback of this method is that the user must set numerous parameters, and the values of those parameters have a large effect on the size of the home range. In particular, it has been shown that even small changes in the smoothing factor (h) can have a large effect on the size of the home range (Worton, 1995). This has led to the conclusion that kernels are more suited to analysis of use rather than estimation of home range size (Harris *et al.,* 1990). Methods for choosing the smoothing parameter have been developed. Least-squared cross vali-

dation (LSCV) has been shown to be the most accurate for most situations and is the most widely used (Worton, 1989; Seaman & Powell, 1996; Stevens & Barry, 2002; Morzillo, Feldhamer & Nicholson, 2003). Although LSCV works relatively well for most types of data, it does not perform well when animals use the same location multiple times or when the data are highly autocorrelated (Worton, 1987; Seaman & Powell, 1996). Milksnakes used the same location multiple times, and thus locations were autocorrelated. Sub-sampling is suggested when data are autocorrelated (Worton, 1987). Milksnakes were located on average every 2–3 d, and the distances they cover when they move indicate that this period is ample time to traverse an entire home range. Therefore, we believe it was the snake's choice to stay at a given location: it was not caused by the impossibility of reaching a new location during the time interval between telemetry locations. Sub-sampling may eliminate autocorrelation in this case, but it would reduce the biological significance of the home range estimate (De Solla, Bonduriansky & Brooks, 1999).

Instead of sub-sampling, we chose to combine the MCP and kernel methods to gain the simplicity of the MCP and the ability to determine intensity of use within the home range afforded by the kernel. Snakes were tracked for a full active season, and therefore the MCP was a measure of the maximum area of the home range for the active season. The kernel density estimators are based on probabilities, and because a probability of presence of 100% is extremely unlikely, the 95% kernel is most often used to calculate the area of the total home range of an animal (Powell, 2000). Therefore, for each individual, we used the MCP as the area of the home range and we adjusted the smoothing parameter (h) until the area of the 95% kernel equalled the area of the MCP (Row & Blouin-Demers, 2006a).

## LAND COVER CLASSIFICATION

We derived land cover classification from high-resolution IKONOS imagery (Space Imaging, Thorton, Colorado, USA) with a pixel size of 4 m. We used GRASS 5.0 (GRASS Development Team, 2003) to georeference and classify the imagery. We classified the image into five habitats: field, open water, forest, marsh, and rocky outcrop. We used approximately 20 training sites per habitat type. We chose training sites that were large, where the habitat type was known from field observation, and where the habitat was homogeneous. The training sites were spread evenly throughout the study area, and we sampled the range of variation present within each habitat type. After the classification was complete, we determined classification accuracy. We generated approximately 20 random locations in each habitat type on the classified image and traveled to those points with a GPS unit to classify the habitat on the ground. The average classification accuracy for all habitats was 90%. Marshes had the lowest classification accuracy (77%).

## MACRO-HABITAT

To determine which habitats milksnakes preferred at the home range scale, we needed to compare the habitat use of the individual to the habitat that was available. To quantify habitat use, we determined the percentage of field, rocky outcrop, marsh, and forest in the 95%, 75%, and 50% kernels. Open water was excluded from the home range and from all the analyses. The habitat was not homogeneous throughout the study area, and therefore we did not want to use the composition of the study area as the available habitat for all individuals. Instead, for each individual, we used the composition of a circle centred on the hibernaculum or the first location (for the single individual for which we did not know the location of the hibernaculum) with a radius equal to the length of the home range (the longest distance from one edge of the home range to the edge diametrically opposed) for that particular snake. We then compared the percentage of use to the percentage of availability of each habitat type.

The proportions of the four habitat types sum to one and, therefore, are not independent. Aitchison (1986) demonstrated that for any component $x_i$ of a composition, the log ratio transformation $y_i = LN(x_i/x_j)$ makes the $y_i$ linearly independent. The results of the analysis are independent of the component chosen as the denominator in the log ratio transformation. Following the methods of Aebischer, Robertson, and Kenward (1993), we calculated the difference ($d$) between use and availability log ratios for each habitat (LN[Field/Marsh]; LN[Rocky Outcrop/Marsh]; LN[Forest/Marsh]). We then determined if habitat use was non-random by comparing the residual matrix of raw sums of squares and cross products, calculated from $d$, to the mean-corrected sums of squares and cross products, also calculated from $d$.

When habitat use was non-random with respect to availability, we created a matrix to establish the rank of each habitat with respect to all other habitats (Aebischer, Robertson & Kenward, 1993). We then used $t$-tests to determine if the differences between the ranks of each habitat type were significant.

We defined an edge as the border between forest and another habitat (field, rocky outcrop, marsh). To determine if milksnakes preferred edges at the home range scale, we calculated the length of edge per hectare in each kernel density estimate. We did the same for the circle of habitat available to each individual. For each individual, we calculated the difference in edge length between use and availability. We used $t$-tests to determine if the difference in length of edge per hectare between use and availability was significantly different from zero.

## MICRO-HABITAT

Each time we located a snake with radio-telemetry, we recorded the habitat type (field, rocky outcrop, forest, marsh). From these data, we calculated habitat use as the percentage of locations in each habitat type for each individual. To determine if milksnakes used habitats non-randomly with respect to availability within their home range, we compared habitat use to habitat availability within the 95% kernel home range with the same method as in the macro-habitat analysis. Because our satellite imagery was unable to identify accurately habitat patches < 15 m in diameter, on the ground we ignored habitat patches < 15 m. As with the macro-habitat analysis, we ranked the habitat types in order of preference when habitat use was non-random.

We also conducted a more detailed habitat characterization at all locations, except when snakes were in buildings or human debris because these locations could not be characterized adequately with our sampling scheme. We also did not characterize locations that were < 10 m from a previously characterized location because 10 m was the largest sampling radius used during characterization. We characterized the habitat approximately 1 week after the snake had moved to a new location to ensure that the snake was not disturbed but that structural changes occurring between use and characterization were minimal. Locations that were used more than once were included only once in the analysis.

To determine if snakes used the habitat non-randomly at this fine scale, we quantified available habitat by repeating the same characterization at paired locations selected at random. Random locations were determined by walking a randomly determined distance (10 to 200 paces, determined by rolling a 20-sided die and multiplying by 10) in a randomly determined direction (0 to 360°, determined by spinning the bearing dial of a compass) from each snake location characterized.

To characterize the habitat, we measured 24 structural variables in circular plots of different radii (Table I). We chose sampling radii based on the smallest possible radius where variation was present and measurable. To evaluate ground cover and canopy closure, we used a sighting tube (50 × 2.5 cm piece of pipe) with a cross wire at one end (Winkworth & Goodall, 1962). We aimed the tube haphazardly at the ground 50 times within a 1-m radius and recorded the number of times each cover type was sighted in the cross hairs. Similarly, to measure canopy closure we lay on the ground (to have the snake's perspective) and aimed the tube haphazardly within a 45° angle cone 50 times and recorded the number of times vegetation above 1.5 m was sighted in the cross hairs. In both cases, we multiplied the number of hits by two to obtain a percentage.

To analyze micro-habitat selection at this scale, we used matched-pairs logistic regression. In the paired design, each snake location is compared to its paired random location to control for variations in environmental conditions and to ensure that the random locations were available to the individual. It was impossible to rule out completely the potential use of the random locations by milksnakes. When use is rare, however, it is acceptable to use a matched-pairs design because it is expected that the random locations will be made up almost exclusively of unused locations (Keating & Cherry, 2004).

In the matched-pairs logistic regression, we subtracted the values for all the habitat variables (Table I) at each random location from the values at each paired snake location. We then used standard logistic regression with the constant term excluded to regress the difference between the presence and absence of snakes (all 1's) against the differences in habitat values between used and random locations. The estimated coefficients are interpreted the same way as in standard logistic regression except they are interpreted as differences in the habitat and not as absolute values (Hosmer & Lemeshow, 2000; Compton, Rhymer & McCollough, 2002).

An assumption of logistic regression is that each observation is independent. This is hard to achieve in radio-telemetry studies where many locations are obtained from few individuals. In this study, we collected habitat-use data on 8 females and 15 males. The range in the number of observations was large for females (range = 5–25, median = 11) and males (range = 2–21, median = 11). Because we had many variables in the model, it would have been impossible to fit individual models for snakes for which we had few locations. Therefore, we used each snake location as an independent location in the model. Since no individual represented a large proportion of the total locations (median = 6.8%, max = 12%), no individual had the opportunity to bias the results unduly.

To select the most parsimonious model, we conducted preliminary univariate and multivariate analyses and eliminated variables that were far from contributing significantly to the difference between used and random locations ($P > 0.25$) (Tables I and II). From the remaining variables, we selected candidate models and compared the models against each other using Akaike's Information Criterion (AIC) (Burnham & Anderson, 1998). We also selected the best model using a stepwise procedure to confirm our best model. The linearity of the final model was tested using

TABLE I. Structural variables used in the analysis of habitat selection by milksnakes in Ontario (2003 and 2004) with associated abbreviations and sampling radii.

| Variable | Radius (m) | Description |
|---|---|---|
| VARIABLES USED IN UNIVARIATE AND MULTIVARIATE ANALYSES | | |
| CANCLO | 15 | Canopy closure (%) within 45º cone |
| LBOULDER | 20 | Length of nearest rock (m) (> 20 cm length) |
| DLOG | 20 | Distance (m) to nearest log (> 7.5 cm diameter) |
| LLOG | 20 | Length (m) of nearest log |
| DUNDER | 20 | Distance (m) to nearest understory tree (> 2 m and < 7.5 cm DBH) |
| DOVER | 20 | Distance (m) to nearest overstory tree (>7.5 cm DBH) |
| DEDGE | 100 | Distance (m) to nearest edge |
| NBOULDER | 5 | Number of rocks (> 20 cm length) |
| NLOG | 5 | Number of logs (> 7.5 cm diameter) |
| NUNDER | 5 | Number of trees (< 7.5 cm DBH and > 2m) |
| N7.5–15 | 10 | Number of trees > 7.5 cm and < 15 cm DBH in plot |
| N15–30 | 10 | Number of trees > 15 cm and < 30 cm DBH in plot |
| N30–45 | 10 | Number of trees > 30 cm and < 45 cm DBH in plot |
| N>45 | 10 | Number of trees > 45 cm DBH in plot |
| %ROCK | 1 | Coverage (%) of rocks within plot |
| %LEAF | 1 | Coverage (%) of leaf litter within plot |
| VARIABLES ELIMINATED AFTER UNIVARIATE ANALYSES ($P > 0.25$) | | |
| %GRASS | 1 | Coverage (%) of grass within plot |
| %SHRUB | 1 | Coverage (%) of shrubs within plot |
| %HERB | 1 | Coverage (%) of herbs (non-woody) within plot |
| %SOIL | 1 | Coverage (%) bare soil within plot |
| %STICK | 1 | Coverage (%) sticks within plot |
| %LOG | 1 | Coverage (%) of logs within plot |
| DIALOG | 20 | Diameter (m) of nearest log (> 7.5 cm diameter) |
| DBOULDER | 20 | Distance (m) to nearest rock (> 20 cm length) |

DBH = diameter at breast height.

TABLE II. Variables included in the multivariate model explaining micro-habitat use by milksnakes in Ontario (2003 and 2004). Variables that explained a significant portion ($P < 0.25$) of the multivariate model were used to derive candidate models.

| Variable | Coefficient | Deviance ($G^2$) | $P$ |
|---|---|---|---|
| CANCLO | -0.02 | 5.48 | 0.02 |
| LBOULDER | 1.59 | 6.35 | 0.02 |
| DLOG | 0.00 | 0.00 | 0.94 |
| LLOG | -0.08 | 1.23 | 0.27 |
| DUNDER | 0.02 | 0.27 | 0.60 |
| DOVER | -0.06 | 1.48 | 0.23 |
| DEDGE | -0.03 | 14.86 | < 0.01 |
| NBOULDER | 0.03 | 10.10 | < 0.01 |
| NLOG | -0.01 | 0.04 | 0.84 |
| NUNDER | 0.00 | 0.12 | 0.73 |
| N7.5–15 | -0.03 | 0.70 | 0.41 |
| N15–30 | -0.01 | 0.01 | 0.92 |
| N30–45 | 0.12 | 1.22 | 0.26 |
| N>45 | -0.01 | 0.25 | 0.62 |
| %ROCK | 0.04 | 9.89 | < 0.01 |
| %LEAF | -0.02 | 3.50 | 0.07 |



FIGURE 1. Mean percentages (± 1 SE) of four habitat types within three densities of kernel home range estimators of milksnakes and percentages of available habitat in the study area in Ontario (2003 and 2004).

design variables based on the quartiles of each variable (Hosmer & Lemeshow, 2000).

### STATISTICAL ANALYSES

Habitat divergence between male and non-gravid female snakes is rare unless significant morphological differences are present between the sexes (Reinert, 1993). Row and Blouin-Demers (2006b) found no differences in thermoregulation, seasonal habitat use, and behaviour between male and non-gravid female milksnakes. Thus, we pooled the sexes for all analyses.

All statistical analyses were performed with R (R Development Core Team, 2004) and JMP version 5 (SAS Institute, 2002). We inspected box plots to determine whether assumptions of normality and homogeneity of variance were upheld. When we found deviations, the variables were transformed to meet the assumptions. We reported all means ± 1 SE and accepted significance of tests at $\alpha = 0.05$.

## Results

### MACRO-HABITAT

We collected data on 15 males and 8 females. For this analysis, however, we used only individuals for which the home range size plotted against the number of locations reached an asymptote. We applied this restriction to ensure that the number of locations was sufficient to estimate total home range size. This limited the sample size to 11 males and 8 females, all of which were located more than 24 times (median = 36, range = 24–48). In all three kernel densities of milksnake home ranges, rocky outcrops and fields were more prevalent than randomly available, whereas marshes and forest were less prevalent than randomly available. The preference for rocky outcrops and fields became stronger as the intensity of use increased (from the 95% kernel to the 50% kernel, Figure 1).

For each kernel density, we calculated a likelihood ratio statistic ($\Lambda$) by dividing the residual matrix of raw sums of squares and cross products by the mean-corrected sums of squar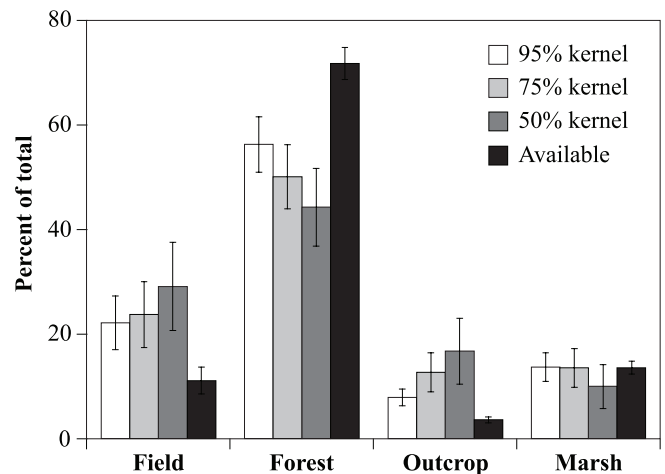es and cross products (Aebischer, Robertson & Kenward, 1993). The distribution of -Nln$\Lambda$ approximates the $\chi^2$ distribution (Aebischer, Robertson & Kenward, 1993). For the 95% kernel home range, the habitat used was significantly different from the habitat available ($\chi^2_4 = 12.49$, $P = 0.01$). For the 75% kernel ($\chi^2_4 = 7.59$, $P = 0.11$) and the 50% kernel ($\chi^2_4 = 6.13$, $P = 0.18$), however, the difference between use and availability was not significant.

For the 95% kernel home range, the preference rankings (from most to least preferred) of the habitats were rocky outcrop > field > > marsh > forest. There were no significant differences between the top two ($d = 0.06$, $t_{14} = 0.25$, $P = 0.80$) or bottom two habitats ($d = 0.18$, $t_{14} = 0.71$, $P = 0.45$).

The 95% kernel milksnake home range ($d = 1.3$, $t_{14} = 5.14$, $P < 0.001$) and the 75% kernel ($d = 66.15$, $t_{14} = 3.30$, $P = 0.004$) contained significantly more edge habitat than available. The 50% kernel also contained more edge than available, but this difference was only marginally significant ($d = 0.51$, $t_{14} = 1.97$, $P = 0.07$).

### MICRO-HABITAT

We used the same snakes here that we used in the macro-habitat analysis (11 males and 8 females) to allow for comparisons. At this scale, milksnakes again used rocky outcrops and fields more than they were available within the 95% kernel home range and forests and marshes less than they were available (Figure 2). Using the same methods as in the macro-habitat analysis, we determined that milksnakes used micro-habitats non-randomly ($\chi^2_4 = 34.30$, $P < 0.001$) and that the order of selection was field > rocky outcrop >> forest >> marsh. There was no significant difference between the two most preferred habitats ($d = 0.50$, $t_8 = 0.97$, $P = 0.60$), however.

### LOGISTIC REGRESSION

We characterized the habitat in detail at 279 snake locations (166 male and 113 female locations) and 279 paired random locations. Because we had many predictor variables, we ran separate univariate models for each variable and eliminated clearly non-significant variables ($P > 0.25$)

(Table I) before our multivariate analysis. We fit a multivariate model with the remaining variables and eliminated variables that did not contribute significantly to the multivariate model ($P > 0.25$) (Table II). From the seven remaining variables, we selected 10 candidate models based on the significance level of each variable (Table III) and compared the models against each other. We chose the model with the lowest AIC value. We then added the variables that were eliminated in preliminary analyses to the best candidate model and ran a backward stepwise procedure (that eliminated variables sequentially based on their AIC values) to ensure that the eliminated variables did not significantly increase model fit. This procedure selected the same model as the model in our original approach. The best model was significant and explained a large portion of the variation ($R^2 = 0.29$, Log-Likelihood$_{6, 278} = 195$, $P < 0.001$). The top two models had similar AIC values and weights, but they shared all but one variable (Table III).

Based on the odds ratios (Table IV), milksnakes used locations with open canopy that were close to edges: a 10% increase in CANCLO resulted in a 18% decrease in the probability of selection, and similarly a 10-m increase in DEDGE resulted in a 26% decrease in probability of selection (Table IV). Milksnakes also demonstrated a strong preference for rocky habitats. A size increase of 10 cm in LBOULDER resulted in an 18% increase in the probability of selection. Increasing NBOULDER by 5 led to a 16% increase in the probability of selection. A 10% increase in %ROCK at the snake location led to a 35% increase in the probability of selection. Finally, milksnakes avoided leaf litter: a 10% increase in %LEAF led to an 18% decrease in the probability of selection.

## Discussion

Milksnakes used the habitat non-randomly at both the macro- and micro-habitat scales. At the macro-habitat scale, milksnakes preferred rocky outcrops and fields to forest and marshes. Milksnakes also preferred home ranges with more edge habitat. Although these trends seemed to be stronger in the core areas of the home ranges (Figure 1), habitat use did not differ significantly from random in the 75% and 50% kernels. At the micro-habitat scale, milksnakes again preferred fields and rocky outcrops to forest and marshes. Milksnakes also preferred locations that were close to edges.

In the thermally challenging environment of our study area, open habitats and edges have environmental temperatures that deviate less from $T_{set}$ (Blouin-Demers & Weatherhead, 2001b; Row & Blouin-Demers, 2006b), and these habitats offer characteristics that benefit thermoregulation (increased solar radiation, wider range of temperatures). Therefore, the fact that milksnakes prefer open habitats and edges at both scales supports our prediction that thermal quality strongly affects habitat selection.

A correlation does not imply causation, however, and other factors known to influence habitat use could be correlated with thermal quality. The main prey of milksnakes are small mammals (Williams, 1988), and in our study area small mammal trapping revealed that prey of milksnakes are more abundant in forest than in fields (Row and Blouin-Demers, 2006b). Few small mammals live in marshes, and this low food abundance could explain why milksnakes avoided marshes even though marshes have high thermal quality: milksnakes may select habitats based on thermal quality, but with the condition that the habitats have some prey. There are a variety of predators of milksnakes in our study area. Although we did not quantify predator abundance in different habitats, it seems unlikely that milksnakes selected open habitats to avoid predators, because there is less cover in open habitats.

With matched-pairs logistic regression at the micro-habitat scale, we confirmed that milksnakes preferred locations with open canopy and avoided forest interior. This again supported our prediction that milksnakes select habitats that facilitate behavioural thermoregulation. Milksnakes also preferred locations close to large rocks and with many boulders. Milksnakes use boulders and rock piles frequently as retreat sites. In direct sun, the operative environmental temperatures are often above the preferred body temperature of snakes (Row & Blouin-Demers, 2006b). By selecting rocky locations in the open, milksnakes have simultane-
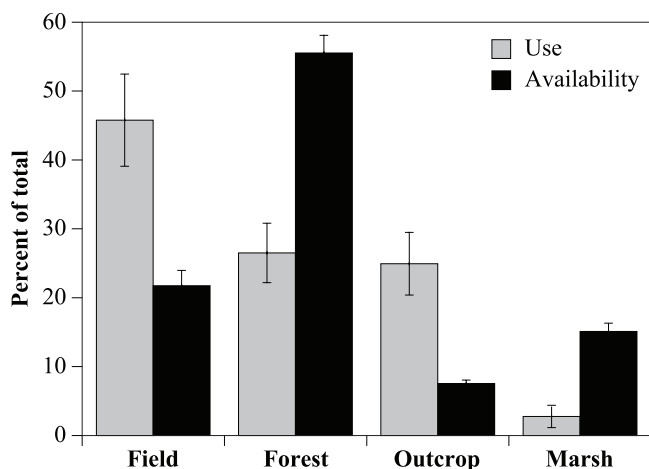
FIGURE 2. Mean percentage of locations (± 1 SE) in each of four habitat types for milksnakes in Ontario (2003 and 2004). Available habitat is the mean percentage of each habitat type (± 1 SE) within the 95% kernel home range estimators.

TABLE III. Top five candidate models, and their relative weights, explaining micro-habitat use by milksnakes in Ontario (2003 and 2004). Candidate models were chosen from significant variables in univariate and multivariate analyses ($P < 0.25$).

| Model | Log-likelihood | AIC | Akaike weight |
|---|---|---|---|
| LBOULDER + NBOULDER + CANCLO + DEDGE + %ROCK + %LEAF | 195 | 204.16 | 0.46 |
| LBOULDER + NBOULDER + CANCLO + DEDGE + %ROCK + %LEAF + DOVERSTORY | 196 | 204.60 | 0.37 |
| LBOULDER + NBOULDER + CANCLO + DEDGE + %ROCK | 190 | 206.52 | 0.14 |
| LBOULDER + NBOULDER + DEDGE + %ROCK + %LEAF + DOVERSTORY | 188 | 210.96 | 0.02 |
| NBOULDER + CANCLO + DEDGE + %ROCK | 182 | 212.97 | 0.01 |

TABLE IV. Coefficients and odds ratios for the best model explaining micro-habitat use by milksnakes in Ontario (2003 and 2004).

| Variable | Coefficient | SE | Increase | Odds ratio | 95% CI (OR) |
|---|---|---|---|---|---|
| LBOULDER | 1.64 | 0.63 | 10 cm | 1.18 | (1.11, 1.25) |
| NBOULDER | 0.03 | 0.01 | 5 boulders | 1.16 | (1.10, 1.22) |
| CANCLO | -0.02 | 0.01 | 10% | 0.82 | (0.74, 0.90) |
| DEDGE | -0.03 | 0.01 | 10 m | 0.74 | (0.67, 0.82) |
| %ROCK | 0.03 | 0.01 | 10% | 1.35 | (1.22, 1.49) |
| %LEAF | -0.02 | 0.01 | 10% | 0.82 | (0.74, 0.90) |

ous access to the open habitat for basking and to numerous retreats for cooling or for protection from predators.

Our study highlights the importance of studying habitat selection at multiple spatial scales to gain a more complete understanding of selection patterns. At different spatial scales, there are often distinct (Orians & Wittenberger, 1991; Luck, 2002) and sometimes conflicting (Compton, Rhymer & McCollough, 2002) habitat selection patterns. It has been suggested that this results from differing selection pressures and limiting factors at various spatial scales (Rettie & Messier, 2000). For milksnakes in our northern study area, the need to thermoregulate seems to be an important factor driving habitat selection at both spatial scales studied. Nevertheless, other potentially influential factors, such as foraging or predator avoidance, could not be excluded formally.

## Conclusion

Many studies have demonstrated the importance of studying habitat selection at multiple spatial scales (Orians & Wittenberger, 1991; Compton, Rhymer & McCollough, 2002; Luck, 2002). In very few studies of organisms other than mammals and birds, however, was second-order selection investigated: selection of a home range within the study area. This is surprising because reptiles, and snakes in particular, are ideal organisms to study with respect to habitat selection. First, unlike mammals and birds that are easily disturbed, snakes can be located within 1 m without disruption. Therefore, they do not have to be located using triangulation, which leads to large errors in position (Rettie & McLoughin, 1999). Second, snakes do not move far and often use the same location more than once. Therefore, locations every 2–3 d throughout the study season are sufficient to ensure that the entire home range is estimated.

In this study, milksnakes preferred habitats with the highest thermal quality. The most parsimonious explanation for this pattern is that snakes use these habitats to facilitate behavioural thermoregulation. Presumably, we found habitat use to be linked strongly to thermal quality because we studied milksnakes in a thermally challenging environment. It would be interesting to study habitat selection at multiple spatial scales in relation to thermal quality in a thermally benign environment. Under these conditions, thermal quality should be less important and other factors, such as foraging or predation risk, should have a greater influence on habitat selection. In addition, the effects of reproductive state, season, and level of disturbance on habitat selection could be examined at multiple spatial scales with sufficient sample sizes.

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