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Thermal quality influences thermoregulation, behaviour and habitat selection at multiple spatial scales
in eastern milksnakes (*Lampropeltis triangulum*)

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**Thermal quality influences thermoregulation, behaviour and habitat selection at
multiple spatial scales in eastern milksnakes (*Lampropeltis triangulum*)**

Jeffrey R. Row

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ABSTRACT

Body temperature variation affects most physiological processes in terrestrial ectotherms and, therefore, can have a large effect on fitness. I studied eastern milksnakes at the northern extreme of their range, in a thermally challenging environment. Because of the particular importance of thermoregulation in thermal extremes, I hypothesized that thermal quality would influence thermoregulation, behaviour and habitat selection.

In chapter one, I demonstrated that when thermal quality decreased, milksnakes increased investment in thermoregulation, a result contrary to the central prediction of the cost-benefit model of thermoregulation. Because ectotherms obtain heat from their environment, I predicted that when thermal quality was low, milksnakes would bask more and select higher thermal habitat. Milksnakes did not alter their habitat use in response to thermal quality. They did, however, alter their behaviour and were seen basking more and concealed less when thermal quality was low.

In chapter two, my main goal was to determine if thermal quality affected habitat selection at multiple scales. Supporting my prediction, thermal quality influenced habitat selection at both the home range scale and the location scale. At both scales, milksnakes selected high thermal quality habitats (rocky outcrops, fields, and edges) and avoided low thermal quality habitat (forest). At the microhabitat scale, milksnakes selected locations with open canopy and many rocks. As a secondary objective, I tested the ability of 4 m resolution IKONOS imagery to accurately quantify habitat selection at the scale of the locations used by each snake. Although not as accurate as traditional field methods, remote sensing showed promise in measuring habitat selection at that scale.

RÉSUMÉ

Chez les ectothermes terrestres, la température corporelle affecte la majorité des processus physiologiques et, conséquemment, a un effet important sur l'aptitude. Par conséquent, les ectothermes devraient thermoréguler afin de maintenir une température corporelle permettant de maximiser la performance. J'ai conduit cette étude sur la couleuvre tachetée de l'est à l'extrême nord de sa répartition, dans un environnement thermique exigeant. Étant donné l'importance de la thermorégulation aux extrêmes climatiques, j'ai prédit que la thermorégulation, le comportement et la sélection d'habitat devraient être influencés par la qualité thermique.

Dans le chapitre un, j'ai démontré que la qualité thermique était supérieure au cours de l'été que durant le printemps et l'automne, et supérieure dans les habitats ouverts (marais, affleurements rocheux et champs) que dans la forêt. Lorsque la qualité thermique décroissait, les couleuvres tachetées augmentaient l'investissement en thermorégulation, une trouvaille contraire au modèle coûts-bénéfices de la thermorégulation. Puisque les ectothermes obtiennent leur chaleur de leur environnement, j'ai prédit qu'un changement d'investissement en thermorégulation se traduirait par des changements de sélection d'habitats et de comportement. Les couleuvres tachetées ne modifiaient pas leur sélection d'habitat en réponse à la qualité thermique. Toutefois, lorsque la qualité thermique était moindre, les couleuvres modifiaient leurs comportements et étaient observées plus fréquemment à découvert.

Dans le chapitre deux, j'ai démontré que la qualité thermique influençait la sélection d'habitat à l'échelle du domaine vital et à l'échelle du site. Aux deux échelles, les couleuvres tachetées sélectionnaient les habitats de qualité thermique supérieure (marais, affleurements

rocheux et écotones) et évitaient les habitats de qualité thermique moindre (forêt). À l'échelle du microhabitat, les couleuvres tachetées, sélectionnaient les habitats avec une canopée ouverte et plusieurs roches. Comme objectif secondaire, j'ai testé l'habileté de l'imagerie IKONOS d'une résolution de 4 mètres à quantifier précisément la sélection d'habitat à l'échelle du site utilisé par chacune des couleuvres. Bien que la télédétection n'est pas aussi précise que les méthodes de terrain traditionnelles, cette technologie est prométeuse pour déterminer la sélection d'habitat à l'échelle du site.

TABLE OF CONTENTS

ABSTRACT	II
RÉSUMÉ	III
TABLE OF CONTENTS	V
LIST OF TABLES	VII
LIST OF FIGURES	IX
ACKNOWLEDGEMENTS	XI
CHAPTER 1	13
INTRODUCTION	14
METHODS	18
<i>Study Area and Study Species</i>	18
<i>Radio Telemetry</i>	18
<i>Body Temperature (T_b)</i>	19
<i>Thermal Preference</i>	19
<i>Accuracy of T_b</i>	20
<i>Operative Environmental Temperatures and Thermal Quality</i>	20
<i>Indices of Thermoregulation</i>	22
<i>Habitat Use and Behaviour</i>	24
<i>Prey Density</i>	25
<i>Statistical Analyses</i>	25
RESULTS	26
<i>Thermal Preference</i>	26
<i>Operative Environmental Temperatures</i>	27
<i>Body Temperatures</i>	29
<i>Effectiveness of thermoregulation ($d_e - d_b$)</i>	29
<i>Habitat Use and Behaviour</i>	30

<i>Movements</i>	31
<i>Prey Density</i>	32
DISCUSSION	32
<i>Habitat Use and Behaviour</i>	34
CHAPTER 2	50
INTRODUCTION	51
METHODS.....	53
<i>Study Area and Study Species</i>	53
<i>Radio-telemetry</i>	54
<i>Home Ranges</i>	55
<i>Land Cover Classification</i>	57
<i>Macrohabitat Selection</i>	57
<i>Microhabitat Characterization</i>	59
<i>Microhabitat Selection Based on Land Cover</i>	60
<i>Microhabitat Selection Based on Habitat Characterization</i>	60
<i>Comparison of Remote Sensing and Traditional Approaches</i>	62
<i>Statistical Analyses</i>	63
RESULTS.....	63
<i>Macrohabitat Selection</i>	63
<i>Microhabitat Selection Based on Land Cover</i>	65
<i>Microhabitat Selection Based on Habitat Characterization</i>	65
<i>Comparison of Remote Sensing and Traditional Approaches</i>	67
DISCUSSION	67
<i>Hierarchical Habitat Selection</i>	67
<i>Remote Sensing</i>	69
<i>Conclusion</i>	71
LITERATURE CITED	85

LIST OF TABLES

TABLE 1-1. MULTIPLE REGRESSION EQUATIONS USED TO PREDICT MODEL SNAKE TEMPERATURES IN DIFFERENT HABITATS AVAILABLE TO EASTERN MILKSNAKES IN ONTARIO.	39
TABLE 1-2. MEAN $\pm$ SE, MAXIMUM, AND MINIMUM OPERATIVE ENVIRONMENTAL TEMPERATURES (T_e) RECORDED IN THE FOUR MAIN HABITATS AVAILABLE TO EASTERN MILKSNAKES IN ONTARIO. MEAN $\pm$ SE, MAXIMUM AND MINIMUM AND PERCENT EQUAL TO ZERO DEVIATIONS OF OPERATIVE ENVIRONMENTAL TEMPERATURES (D_e) FROM T_{set} OF EASTERN MILKSNAKES.	40
TABLE 2-1. ACCURACY OF CLASSIFICATION FOR EACH HABITAT TYPE BASED ON LOCATIONS SELECTED ON THE IMAGERY THAT WERE VERIFIED ON THE GROUND. CLASSIFICATION WAS PERFORMED ON IKONOS IMAGERY WITH 4 M RESOLUTION.	72
TABLE 2-2. OUTLINE OF THE MATRIX USED TO COMPARE HABITAT USE BETWEEN HABITAT TYPES AND ESTABLISH HABITAT RANKINGS. THE NUMBER OF POSITIVE VALUES IN EACH ROW RANKS THE HABITAT IN INCREASING ORDER OF RELATIVE USE. MODIFIED FORM AEBISCHER ET AL. (1993).	73
TABLE 2-3. STRUCTURAL VARIABLES USED IN THE ANALYSIS OF HABITAT SELECTION BY EASTERN MILKSNAKES IN ONTARIO (2003 AND 2004) WITH ASSOCIATED ABBREVIATIONS AND SAMPLING RADII.	74
TABLE 2-4. MATRICES OF T-VALUES AND ASSOCIATED P-VALUES COMPARING USE BETWEEN PAIRS OF HABITAT TYPES FOR 3 KERNEL HOME RANGE ESTIMATORS GENERATED FROM EASTERN MILK SNAKE LOCATIONS IN ONTARIO (2003 AND 2004). PREFERENCE RANKINGS ARE IN ORDER OF MOST (1) TO LEAST PREFERRED (4).	76
TABLE 2-5. MATRICES OF T-VALUES AND ASSOCIATED P-VALUES COMPARING USE BETWEEN PAIRS OF HABITAT TYPES FOR EASTERN MILK SNAKE LOCATIONS IN ONTARIO (2003 AND 2004). PREFERENCE RANKINGS ARE IN ORDER OF MOST (1) TO LEAST (4) PREFERRED.	78
TABLE 2-6. PAIRED LOGISTIC REGRESSION MODELS OF MICROHABITAT USE BY EASTERN MILKSNAKES IN ONTARIO (2003 AND 2004).	79
TABLE 2-7. PAIRED LOGISTIC REGRESSION MODELS FOR MALE AND FEMALE MILKSNAKES IN ONTARIO (2003 AND 2004).	80

TABLE 2-8. MATRICES OF T-VALUES AND ASSOCIATED P-VALUES COMPARING USE BETWEEN PAIRS OF HABITAT TYPES FOR EASTERN MILK SNAKE LOCATIONS IN ONTARIO (2003 AND 2004). LOCATION DATA WAS COLLECTED USING 10 M BUFFERS COLLECTED BY REMOTE SENSING. PREFERENCE RANKINGS ARE IN ORDER OF MOST (1) TO LEAST (4) PREFERRED.	81
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LIST OF FIGURES

FIG. 1-1: MEAN OPERATIVE ENVIRONMENTAL TEMPERATURES ($T_e \pm 1$ SE) FOR ALL HABITATS IN 2003 AND 2004 ARE LOWER IN THE SPRING AND FALL THAN IN THE SUMMER.....	41
FIG. 1-2: MEAN OPERATIVE ENVIRONMENTAL TEMPERATURES ($T_e \pm 1$ SE) FOR EACH HOUR OF THE DAY IN FOUR DIFFERENT HABITATS IN THE A) SPRING, B) SUMMER, AND C) FALL IN ONTARIO. THE RANGE OF PREFERRED BODY TEMPERATURE (T_{set}) IS REPRESENTED BY THE SOLID BLACK LINES.	42
FIG. 1-3: WEIGHTED LEAST SQUARE MEAN (WEIGHTED BY SAMPLE SIZE AND CORRECTED FOR SEX) BODY TEMPERATURE ($T_b \pm 1$ SE) IN EASTERN MILKSNAKES IN DIFFERENT HABITATS AND SEASONS. OPERATIVE ENVIRONMENTAL TEMPERATURES ($T_e \pm 1$ SE) WERE HIGHER IN OPEN HABITATS THAN FORESTS AND IN THE SUMMER THAN THE SPRING AND FALL IN ONTARIO IN 2003 AND 2004.	43
FIG. 1-4: WEIGHTED LEAST SQUARE MEAN (WEIGHTED BY SAMPLE SIZE AND CORRECTED FOR SEX) THERMOREGULATORY EFFECTIVENESS INDEX ($D_e - D_b \pm 1$ SE) SHOWING A HIGHER INVESTMENT IN THERMOREGULATION IN FORESTED HABITATS THAN OPEN HABITATS IN ALL THREE SEASONS. CALCULATED BY THE OPPORTUNITIES OFFERED IN EACH SEPARATE HABITAT DURING THE DAY (08:00-16:00) IN ONTARIO. MEAN DEVIATIONS OF OPERATIVE ENVIRONMENTAL TEMPERATURES FROM T_{set} IN EASTERN MILKSNAKES SHOWING A HIGHER THERMAL QUALITY IN OPEN HABITATS IN ONTARIO IN THREE DIFFERENT SEASONS.	44
FIG. 1-5: EASTERN MILKSNAKES THERMOREGULATE MORE EFFECTIVELY (HIGH $D_e - D_b$) WHEN THE THERMAL QUALITY OF THE HABITAT (D_e) IS LOW DURING THE DAY (0800-1600) IN ONTARIO. THIS IS INDICATED BY THE MEAN SLOPE (DASHED LINE) BEING SIGNIFICANTLY LESS THAN 1 (THERMOCONFORMITY).	45
FIG. 1-6: MEAN PERCENTAGE OF OPEN HABITAT ($\% \pm 1$ SE) IS CONSISTENTLY HIGHER IN EASTERN MILK SNAKE LOCATIONS THAN IN RANDOM LOCATIONS ACROSS ALL THREE SEASONS IN ONTARIO IN 2003 AND 2004.....	46
FIG. 1-7: A HIGHER PERCENTAGE OF EASTERN MILKSNAKES WERE CONCEALED AND A LOWER PERCENTAGE WERE BASKING WHEN LOCATED IN THE SUMMER AND FALL THAN THE SPRING IN ONTARIO IN 2003 AND 2004.	47
FIG. 1-8: EASTERN MILKSNAKES HAVE A HIGHER MEAN RATE OF MOVEMENT (DISTANCE TRAVELED PER DAY) IN THE SUMMER THAN IN THE SPRING AND FALL IN ONTARIO IN 2003 AND 2004.....	48
FIG. 1-9: MEAN PREY DENSITY (MICE/TRAP * 100 NIGHTS ± 1 SE) OF EASTERN MILKSNAKES IS HIGHER IN FORESTED HABITATS IN THE SUMMER AND FALL BUT HIGHER IN OPEN HABITATS IN THE SPRING IN ONTARIO.....	49

FIG. 2-1. PERCENTAGE OF FOUR HABITAT TYPES WITHIN VARYING KERNEL HOME RANGE ESTIMATORS AND AVAILABLE HABITAT FOR EASTERN MILKSNAKES IN ONTARIO (2003 AND 2004).....	82
FIG. 2-2. MEAN PERCENTAGE OF LOCATIONS ($\pm$ SE) IN EACH OF FOUR HABITAT TYPES FOR A) MALE AND B) FEMALE MILKSNAKES IN ONTARIO (2003 AND 2004) BASED ON FIELD SURVEYS. AVAILABLE HABITAT IS THE MEAN PERCENTAGE OF EACH HABITAT TYPE ($\pm$ SE) WITHIN THE 95% KERNEL HOME RANGE ESTIMATORS.....	83
FIG. 2-3. MEAN PERCENTAGE OF 10 M BUFFERS ($\pm$ SE) IN EACH OF FOUR HABITAT TYPES FOR A) MALE AND B) FEMALE MILKSNAKES IN ONTARIO (2003 AND 2004) BASED ON SATELLITE IMAGERY. AVAILABLE HABITAT IS THE MEAN PERCENTAGE OF EACH HABITAT TYPE ($\pm$ SE) WITHIN THE 95% KERNEL HOME RANGE ESTIMATORS.....	84

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CHAPTER 1

Thermal quality influences investment in thermoregulation, habitat use and behaviour in eastern milksnakes (*Lampropeltis triangulum*)

Introduction

Body temperature variation affects most physiological and developmental processes in terrestrial ectotherms (Peterson et al. 1993) and, therefore, has a large effect on fitness (Christian and Tracy 1981, Huey and Kingsolver 1989). Ectotherms should thus thermoregulate to maintain body temperatures (T_b) within an optimal range that maximizes performance and, therefore, fitness (Christian and Tracy 1981, Huey and Kingsolver 1989). The extent of thermoregulation, however, varies by species and by environment. Some species are thermoconformers that are active under a broad range of T_b (Ruibal 1961, Hertz 1992) while others are precise thermoregulators that maintain T_b within a narrow range (Adolph 1990). There are costs and benefits to thermoregulation and variation in the ratio of costs to benefits is believed to account for the variation in thermoregulation strategy (Huey and Slatkin 1976). The benefits of thermoregulation come from the increased metabolic and physiological performance realized when T_b is maintained within the optimal range. Because ectotherms regulate T_b through behavioural mechanisms, the associated costs are related to the energy and time that must be invested in seeking the appropriate microclimates. This is energy and time that could be spent on other activities such as foraging or mate searching. Also, basking to increase T_b exposes ectotherms to predators.

Huey and Slatkin (1976) attempted to predicted extent of thermoregulation based on the associated costs and benefits. One important prediction of their model was that ectotherms should invest less in thermoregulation when the thermal quality of their environment is low. In low thermal quality environments, temperatures are far from the optimal range and the time and energy costs associated with thermoregulation are high. Thus, they predicted ectotherms would thermoregulate less precisely in these environments. The

evidence supporting this prediction of the model has been mixed. Some studies conducted in thermally benign environments supported the prediction (Lee 1980, Hertz and Huey 1981), but studies conducted in temperate climates (Blouin-Demers and Weatherhead 2001b, 2002) and a comparative study at the global scale (Blouin-Demers and Nadeau 2005) have found patterns opposite to this prediction. In thermally challenging climates, ectotherms invest more in thermoregulation than in thermally benign climates, probably because the disadvantages of thermoconformity are much higher when the thermal quality is low than when it is high (Blouin-Demers and Weatherhead 2001b, Blouin-Demers and Nadeau 2005). I investigated patterns of thermoregulation and habitat use in eastern milksnakes (*Lampropeltis triangulum*) in a thermally challenging environment. The study area is at the northern extreme of the range of the species and approximately 700 km from the northern limit of reptile distributions in eastern North America. My first goal was to test the prediction that, contrary to the cost benefit model, milksnakes would invest more in thermoregulation when the thermal quality of their environment is low.

The study area is temperate and, therefore, in the spring and fall, temperatures are lower and more variable than in the summer: from an ectotherm's perspective, thermal quality is highest in the summer because the challenge faced by ectotherms in temperate climates is to increase T_b . Thus, I predicted that milksnakes should invest more in thermoregulation in the spring and fall than in the summer. I expected forests to have lower thermal quality than open habitats because little solar radiation reaches the ground in forests. Therefore, I predicted that milksnakes would invest more in thermoregulation while in forests than while in open habitats.

Because terrestrial ectotherms are dependent upon obtaining heat from the physical environment, reptiles must regulate T_b through behaviour and choice of microclimate (Cowles and Bogert 1944, Huey and Kingsolver 1989, Huey 1991, Hertz et al. 1993, Blouin-Demers and Weatherhead 2001b, 2002). This dependency upon the environment for heat has lead to the belief that thermoregulation is tightly linked to habitat selection and behaviour (Reinert 1993, Blouin-Demers and Weatherhead 2002). Empirical data clearly linking the two, however, remain scant. Ectotherms have been shown to modify habitat selection when facing varying temperatures (Christian et al. 1983, Shine and Lambeck 1990, Christian and Bedford 1995, Heard et al. 2004). None of these studies, however, have demonstrated a clear link between changes in habitat selection and thermal quality of the habitat and none were conducted in temperate climates where the link between thermoregulation and habitat use should be particularly tight (Christian and Bedford 1995). Therefore, as a secondary objective, I wanted to determine if milksnakes altered habitat use and behaviour in response to thermal quality. Thus, I tested the prediction that milksnakes should use habitats of higher thermal quality in the spring and fall when the environment is more thermally challenging.

In addition to altering habitat selection, I predicted that milksnakes should be seen basking less often in the summer. Because thermoregulation only requires short movements (shuttling between sun and shade) while most other activities, such as foraging and mate searching, require more extensive movements, I predicted that milksnakes should also, move less, and travel more in the spring and fall.

Other major factors contributing to the habitat selection of terrestrial squamates are sex (Shine and Lambeck 1985, Reinert 1993), reproductive condition (Reinert 1984, 1993, Blouin-Demers and Weatherhead 2001a), prey abundance (Reinert 1993, Madsen and Shine

1996), and predator avoidance. To eliminate these as factors, I tested whether they also affected habitat selection.

The diet of eastern milksnakes is comprised mainly of small mammals (Williams 1988). In the same study area, Blouin-Demers and Weatherhead (2001) found no monthly differences in ratsnake mammalian prey abundance and the only difference between habitats was in August when open habitats had a lower prey abundance than forests and edges. I reanalyzed their data excluding prey that are not consumed by milksnakes (i.e., chipmunks) to ensure that variations in milksnake prey density cannot explain habitat selection.

Differences in habitat selection between male and non-gravid female snakes rarely occurs unless there are morphological differences between the sexes (Reinert 1993). There is sexual size dimorphism in eastern milksnakes with males being slightly larger than females. I did not expect this small difference in size to result in changes of habitat selection or thermoregulation, but I included sex as a factor in all my analyses to control for its potential effects. Reproductive condition, however, has been shown to alter habitat selection and behaviour of many snakes (Reinert 1984, 1993, Blouin-Demers and Weatherhead 2001b). In my study, few data were collected for gravid females and, therefore, they were excluded.

Differences in habitat selection, due to predator avoidance, is harder to determine. The most effective way for milksnakes to avoid predators is to use concealment. I examined the proportion of time when snakes were concealed in different habitats as an indirect test of whether predator avoidance was influencing habitat use.

The main objective of this chapter was to determine if milksnakes invest more in thermoregulation when the thermal quality of the environment is low; a result contrary to the cost benefit model of thermoregulation. As a secondary objective, I attempted to establish if

thermal quality was influencing habitat use and behaviour by determining if milksnakes basked more and used higher thermal quality habitat when thermal quality was low.

Methods

Study Area and Study Species

I conducted this study from August 2002 to November 2004 at the Queen's University Biological Station, 150 km south of Ottawa in eastern Ontario (44°34' N, 76°19' W). The study area was approximately 8 x 3 km and comprised mainly of second growth deciduous forest with numerous granite rocky outcrops, small lakes, and marshes. The abandonment of marginal farmland has also resulted in old fields and scrub habitats being common. Eastern milksnakes seem to exist at low density, but are relatively common throughout the study area.

Radio Telemetry

I captured experimental animals at black ratsnake hibernacula monitored during spring emergence or caught opportunistically throughout the active season. Hibernacula were monitored by surrounding them with plastic fencing fitted with funnel traps (Blouin-Demers et al. 2000a). After capture, I probed the cloaca of the snakes for the presence of hemipenes to determine sex, I measured their snout-vent length (SVL) with a flexible measuring tape, weighed them with an electronic scale, and marked them by injecting a passive integrated transponder (PIT tag) subcutaneously.

From all captured individuals, I selected a subset of animals that were large enough (at least 130 g) to bear the radio-transmitter (Model SB-2T, battery life of 12 months at 20°C, Holohil Systems Inc., Carp, Ontario). The transmitter weighed 5.5 grams, thus never exceeded 5% of the snakes' mass. I aimed at implanting an equal number of males and

females, but this was not possible because females are smaller than males and because I captured fewer females than males. Transmitters were implanted under sterile conditions and isoflurane anaesthesia (Blouin-Demers et al. 2000b).

I released the snakes at the point of capture one day after implantation and located them every 2-3 days using a telemetry receiver (Wildlife Materials, Murphysboro, IL, Communications Specialists, Orange, CA, or AVM instruments, Colfax, CA) and a directional Yagi antenna. Upon location, I recorded the Universal Transverse Mercator (UTM) coordinates with a GPSmap76 (Garmin International Inc., Olathe, KS), the position, and the behaviour of the snake. I used the UTM coordinates to calculate distances moved between locations.

Body Temperature (T_b)

Radio transmitters that were inserted into the body cavity of the snakes emitted a pulse rate that was proportional to temperature. I determined snake T_b by measuring the time for 10 pulses. Snakes were located when they are most active (08:00 – 18:00) and each time the pulse rate was measured to obtain T_b . Taking spot T_b s during day time hours has been shown to over estimate monthly estimates of T_b (Taylor et al. 2004). Seasonal trends in T_b , however, were similar to trends observed using semi-continuous sampling where T_b was taken over longer periods of time (Taylor et al. 2004). In this study, I computed trends to compare thermoregulatory effort of my study snakes between the different seasons and habitats, and not to compare their thermoregulation to values from the literature.

Thermal Preference

When studying thermoregulation, the first step is to determine the preferred T_b range of the organism (T_{set}). T_{set} is believed to match the optimal temperature (T_o) for performance

(Angilletta et al. 2002, Blouin-Demers et al. 2003). T_{set} is most often determined by measuring the T_b selected by individuals in an environment where there are no thermoregulatory costs (Huey 1991, Hertz et al. 1993). To do this, I measured T_b in a thermal gradient in the laboratory. The chamber was a plywood box about 175 cm x 76 cm divided into 4 sections about 43 cm wide. A gradient of approximately 10 – 40 ° C was achieved by placing a piece of flat sheet metal on top of a cool water coil under one end of the box and a piece of sheet metal over heating pads at the other end of the box. Constant and homogeneous illumination was provided by a fluorescent light. I placed radio-implanted individuals into the chamber for 24 hours to become accustomed and then recorded their T_b every 15 min between 6:00 and 22:00. I used the bounds of the central 50% of the observed T_b to determine T_{set} (Hertz et al. 1993, Christian and Weavers 1996, Blouin-Demers and Weatherhead 2001b).

Accuracy of T_b

Following Hertz et al. (1993), I measured the accuracy of T_b (d_b) in the field by calculating the mean deviations, in absolute value, of T_b from T_{set} . When T_b was above T_{set} the difference was calculated as the difference between the T_b and the upper bound of T_{set} . When T_b was below T_{set} the difference was calculated using the lower bound.

Operative Environmental Temperatures and Thermal Quality

Operative environmental temperatures (T_e) refer to the T_b available to the ectotherm in the field. These temperatures can be determined by placing models with similar thermal characteristics as the ectotherm in the various habitats available. Using these models and concurrent environmental conditions, one can build predictive equations that determine the

T_b available to the ectotherm in various habitats at all times, thus providing the T_b that a non-thermoregulating (behaving randomly with respect to thermal quality) animal would achieve. Similar to d_b , the thermal quality of the habitat (d_e) was measured by calculating the absolute value of the mean deviations of T_e from T_{set} .

Models were made from 1.9 cm diameter copper pipe cut to 30 cm and painted with ruddy brown paint to approximate the reflectance of eastern milksnakes (Peterson et al. 1993). I sealed one end of the model by soldering a copper cap and then placed an iButton ($\pm 1^\circ\text{C}$) temperature data logger (Dallas semiconductor Corp., Dallas, Texas) inside to record temperature. The other end of the model was sealed with a rubber stopper and silicone. I calibrated the models by placing a model beside a fresh road kill milksnake (190 g), with an iButton inside the body cavity. The model and the snake were placed in the open for 2 sunny days and the iButtons recorded temperature every 10 minutes.

To record environmental temperatures, I placed the models in 10 microhabitats in July and August. I aimed at measuring the thermal quality of the various microhabitats available to the snakes in open habitats and in forests. I placed four models in the forest; one in the open on the ground and three in shelters (under leaves, under rocks, and under logs). I placed 6 models in open habitats: three in shelters (under grass, under rock, and under log) and three in the open (field, rocky outcrop on grass, rocky outcrop on rock).

The models recorded temperature in all microhabitats every 30 min for 5 weeks (2048 readings). To determine T_e at all times for all microhabitats, I derived multiple regression equations to predict the model temperature from 5 climatic variables recorded by the weather station at the field station (temperature, radiation, wind speed, soil temperature, and rain fall). I used the combination of variables that gave the highest adjusted R^2 value. Then, I used the

predictive equations to calculate T_e for all microhabitats every 30 min for the duration of the active season in both years. I wanted to quantify the thermal quality of the various microhabitats and to calculate a mean T_e for all microhabitats available to the snakes. I grouped all models into one of four major habitat types: forested habitat, forested habitat in shelter, open habitat, and open habitat in shelter. For each of these four habitats, I averaged the T_e of all the models for each hour. I then calculated the mean T_e of the four habitats for each hour, under the assumption that snakes have access to all four habitats. Because the scale of daily movement by milksnakes (130m per move) is much larger than the spatial scale at which those habitats occur (78 m mean diameter if assumed to be a circle), this seems like a reasonable assumption. I used the same method to calculate mean d_e .

Because snakes were tracked once every 2-3 days, I felt it was more appropriate to use only the T_e s when the individual was located as a measure of the thermal environment available to the individual. Therefore, I calculated the mean T_e and the mean d_e for the hour during which each individual was located. Thus, for each snake location, I had a T_b and associated T_e and d_e . I used these values to calculate mean T_e and d_e available for each snake.

Indices of Thermoregulation

Numerous indices of thermoregulation have been developed to measure the extent of thermoregulation. Hertz et al. (1993) emphasized the importance of comparing the extent to which a study animal maintains its T_b within T_{set} , to the degree to which the habitat allows for T_b to be within T_{set} .

Using d_b and d_e , I calculated the effectiveness of thermoregulation with the difference between d_e and d_b (Blouin-Demers and Weatherhead 2001b). This is an open-ended scale that measures the departure of animals from thermoconformity. Thus, negative numbers represent

animals that avoid thermally favourable habitats, zero represents thermoconformity, and positive numbers represent animals that thermoregulate to some degree. The magnitude of the departure from thermoconformity is a measure of thermoregulation effectiveness.

The effectiveness of thermoregulation represents the extent to which individuals exploit the thermal habitats available to them. Thus, when calculating the index to examine thermoregulation differences between seasons, I assumed that all habitats were available to the snakes and I averaged d_e for all habitats. When calculating the index to determine effectiveness of thermoregulation in different habitats, however, I only wanted to know the extent to which the individual was exploiting the thermal opportunities available in that particular habitat. Thus, in that instance I calculated d_e using temperatures available in that habitat only, ignoring temperatures that might have been available in other habitats. I used ANOVAs to determine if investment in thermoregulation was different between open and forested habitats and if investment varied between the seasons.

By determining if milksnakes alter their effectiveness of thermoregulation in different seasons or habitats, I am establishing if broad trends in thermal quality will result in changes in thermoregulatory investment. I wanted to investigate also whether snakes respond to thermal quality on a shorter time scale; do they respond to daily variation in thermal quality. Therefore, for each individual snake I regressed all values of accuracy of T_b (d_b) on their associated values of thermal quality (d_e) and I examined the distribution of slopes for all snakes. I used this regression to avoid the problem of regressing effectiveness of thermoregulation ($d_e - d_b$) on d_e where d_e would be represented on both axes. It can be shown mathematically that testing for a slope of 1 while regressing d_b on d_e is equivalent to testing for a slope of 0 while regressing $d_e - d_b$ on d_e (Blouin-Demers and Nadeau 2005). Therefore, a

slope of > 1 would indicate that thermoregulatory effort decreases when the thermal quality of the environment decreases, which is what is predicted under the cost-benefit model of thermoregulation. A slope of < 1 , however, would demonstrate that effort increases as thermal quality decreases. I used a t-test to determine if the mean of the slopes was significantly different than 1.

Habitat Use and Behaviour

To measure habitat use, each snake location was classified as being in open (field, rocky outcrop, marsh) or forested habitat. The classification of locations was done with ARCVIEW 3.2 (ESRI 2000) using a classified 4 m resolution IKONOS image of the study area (see Chapter 2). Any location in the forest, but within 10 m of an edge with an open habitat, was classified as being in the open habitat because temperature and radiation have been shown to have significant effects on the microclimate at least 10 m into forest (Matlack 1993, Murcia 1995). Also, the accuracy of the GPS unit and the accuracy of the satellite image were also 5 m, combining for a maximum error of 10 m. The average polygon size for forests was 10.2 ha (diameter of 360 m if circular) and 2819 m² (diameter of 60 m if circular) for open habitats and, therefore, the vast majority of polygons were larger than 10 m. Without this buffer, approximately 50% of the edge locations would be placed into the forest due to positional error. I generated a set of random locations for each individual snake to quantify habitat availability. To obtain the random locations, I used the chronological series of distances traveled by each individual, but I used a randomly determined bearing between each move. Thus, the distance between each random location was equal to the distance traveled by the snake between each successive location, but the bearing was different. This approach ensured that all the random locations were available to that particular snake. Each

random location was classified as being in open habitat or forest. To establish if habitat use varied with thermal quality, I used an ANOVA to determine if the difference in percentage of open habitat between snake and random locations varied between seasons.

Each snake location was also classified in terms of the observed behaviour of the snake. I classified the behaviour as basking/resting if the snake was seen immobile, traveling if the snake was moving when located, or concealed if I could not see the snake.

Prey Density

To quantify mammalian prey density in different habitats Blouin-Demers and Weatherhead (2001a) captured small mammals live from 1997 to 1999 using 45 live mouse traps (model 101, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) baited with peanut butter and provided with cotton as bedding. They placed the traps 10 m apart in lines of 15 traps. They placed the trap lines in the forest 30 m from an edge and in rocky outcrops and fields 30 m from an edge. The traps were checked every morning and the number and type of mammals caught were recorded. The original study wanted to quantify prey for black ratsnakes, which are larger than milksnake prey. I removed prey species not consumed by milksnakes (chipmunks) and indexed the abundance of small mammals. I took the average number of captures per trap for one hundred trapping days in each habitat for each season as an index of prey density. To determine if prey density was also influencing habitat selection I used an ANOVA to determine if prey density varied between habitats and seasons.

Statistical Analyses

Multiple locations and T_b were recorded for each individual and were not independent. Therefore, I averaged across individuals for the period being examined (e.g., season) before conducting statistical tests. Individuals that were tracked more than one year

were considered independent from one year to the next, since environmental variables changed from year to year and females could be gravid one year and not in another. Because not all individuals were tracked during the same time period, it was not practical to use repeated measures analysis. Unless otherwise stated, I used the number of times tracked in the period examined as a weighting variable to give more weight to individuals that had been tracked more often and, thus, for which the averages were more accurate. All statistical analyses were repeated without the weighting variable and in all but one case, this did not change the conclusion of the test. If all the interactions in multiway ANOVA's were not significant, they were removed from the model and the analysis was run again, in which cases I present only the results of the reduced model.

I used JMP Version 5.0.1 (SAS Institute 2002) on a Macintosh computer for all statistical analyses. I inspected box plots to determine whether assumptions of normality and homogeneity of variance were upheld. Unless otherwise stated, I detected no significant violations of these assumptions. When I found deviations, the variables were transformed to meet the assumptions. I reported all means $\pm$ one standard error. I accepted significance of tests at $\alpha=0.05$.

Results

I located and measured the T_b of 25 individuals (17 males, 8 females) 890 times over two active seasons (May to October of 2003 and 2004). I located all snakes during the day (8:00-18:00) when the snakes are most active.

Thermal Preference

In total, 4 males, and 3 females were placed in the thermal gradient. For each individual, I calculated the mean T_b and the 75% and 25% quartiles of the distribution of

selected T_b . Averaged across all individuals, mean T_b was $29.9^{\circ}\text{C} \pm 0.53$ and the mean 75% and 25% quartiles were $29.0^{\circ}\text{C} \pm 0.70$ and $31.0^{\circ}\text{C} \pm 0.50$, respectively. One-way ANOVA's showed no differences for the mean ($R^2 = 0.23$, $F_{1,6} = 1.52$, $P = 0.28$), 75% quartile ($R^2 = 0.32$, $F_{1,6} = 2.34$, $P = 0.19$) or 25% quartile ($R^2 = 0.12$, $F_{1,6} = 0.70$, $P = 0.44$) between males and females. Therefore, in subsequent analyses I used the 25% (29.0°C) and 75% (31.0°C) quartiles pooled over the sexes as a measure of T_{set} .

Operative Environmental Temperatures

Temperatures of the copper model and of the 190 g road kill milksnake were highly correlated ($R^2 = 0.97$, $F_{1,604} = 18526.97$, $P < 0.0001$). The temperature of the model and that of the snake, however, were significantly different (mean difference in absolute value = $0.39 \pm 0.64^{\circ}\text{C}$, paired $t_{601} = 14.9$, $P = < 0.001$) indicating that the model tended to slightly underestimate snake temperature. The accuracy of the iButtons used to measure temperature was $\pm 1^{\circ}\text{C}$ and, therefore, this difference was less than the measurement error and was ignored in subsequent analyses.

I derived multiple regression equations to predict model temperatures in different microhabitats using 5 environmental variables. All equations explained a large portion of the variation (mean $R^2 = 0.88$, range = $0.77 - 0.94$) (Table 1-1). I used the equations to determine the T_e and d_e for each hour of the day (8:00-18:00) in all microhabitats from May to October in 2003 and 2004. To divide the active period into three seasons, I averaged T_e in all habitats every 10 days. I divided the active season into spring, summer, and fall by trying to maximize the temperature difference between the seasons and by trying to keep sample sizes similar. As expected, the temperatures in the spring (15 May to 27 June) and fall (16 August to 5 October) are similar and much lower than in the summer (Fig. 1-1).

In all seasons, T_e can fluctuate widely during a day (Fig. 1-2). On average, the only habitat in any season where a snake would be able to reach the lower bound of T_{set} was in open habitats (in direct sunlight). For most of the day in direct sunlight, the snake's T_b would be above the upper bound of T_{set} . In shelters in open habitats, however, T_e never rose above the upper bound of T_{set} and, by shuttling between shelter and direct sunlight a snake could achieve T_{set} for most of the day in any of the seasons in open habitats. In forests this would not be possible because, on average, none of the microhabitats reached the lower bound of T_{set} .

I used mean d_e and percent of time d_e was 0 in each season as a measure of thermal quality (Table 1-2). In all habitats except open habitats in direct sunlight, mean d_e was lower in the summer than in the spring and fall. Sheltered habitats in the open and forests without shelter had the lowest mean d_e 's. The percentage of time d_e was 0 was highest in the summer for all habitats except open habitats in direct sunlight. Open habitats without shelter had the highest percentage with $d_e = 0$ of any habitat in all three seasons. On average, this was the only habitat that reached the lower bound of T_{set} , but T_e in this habitat is above T_{set} for a large part of the day (Fig. 1-2).

Overall, open habitats had a lower mean d_e , a higher percentage of time with $d_e = 0$ and a wider range of temperatures available. These trends were consistent across all three seasons. Therefore, I conclude that open habitats had a higher thermal quality than forest in all three seasons. The difference in thermal quality between habitats, however, was lower in the summer than in the spring and fall. Also, in the spring and fall thermal quality in general was lower than in the summer for both open and forested habitats.

Body Temperatures

I used a 3-way ANOVA with sex as a control variable to determine if T_b varied with season and with habitat type. None of the interaction terms were significant and, therefore, they were removed from the final model. T_b did not vary with sex ($F_{1,72} = 0.33$, $R^2 = 0.32$, $P = 0.57$), but did vary with season ($F_{2,72} = 22.71$, $R^2 = 0.32$, $P < 0.001$) and type of habitat ($F_{1,72} = 7.70$, $R^2 = 0.32$, $P = 0.0065$). Milksnakes had higher T_b 's in open habitats and Tukey-Kramer HSD tests revealed that milksnakes had higher T_b 's in the spring and summer than in the fall (Fig. 1-3).

Effectiveness of thermoregulation ($d_e - d_b$)

I calculated the effectiveness of thermoregulation ($d_e - d_b$) to quantify the thermoregulatory investment by milksnakes in each habitat, in each season. I used a 3-way ANOVA (with sex as a control variable) to determine if effectiveness of thermoregulation differed by season and differed in open and forested habitats. There was a significant interaction between the type of habitat and season ($F_{2,114} = 3.57$, $R^2 = 0.43$, $p = 0.032$). This interaction was not significant when the analysis was not weighted by sample size, but I am more confident in the results with the weighting variable. In the spring, milksnakes had the highest investment in thermoregulation in forested habitats ($t_{38} = 2.43$, $d = 1.20$, $p = 0.020$). In the summer, the investment in thermoregulation decreased in both open and forested habitats, but decreased less in open habitats resulting in no significant difference between open and forested habitats ($t_{43} = 0.70$, $d = 0.41$, $p = 0.48$). In the fall, thermoregulatory investment decreased, but decreased more in open habitats, leading again to significantly more investment in thermoregulation in forest than in open habitats ($t_{35} = 4.20$, $d = 3.14$, $p = 0.0002$) (Fig. 1-4).

When calculating d_e in the previous analysis, I was interested in the effectiveness of thermoregulation in two different habitats. Therefore, when calculating d_e in each habitat I assumed that only temperatures in that habitat were available to the snakes. To determine if milksnakes were investing differently in thermoregulation in each season overall, I used a 2-way ANOVA (with sex as a control variable) and calculated d_e from temperatures that were available to them in all habitats. Effectiveness of thermoregulation varied significantly between seasons ($F_{2,69} = 18.93$, $R^2 = 36$, $p < 0.0001$), but not between the sexes ($F_{1,69} = 0.23$, $R^2 = 0.46$, $p = 0.50$). Tukey-Kramer HSD tests revealed that milksnakes thermoregulated more effectively in the spring than in the summer, and in the summer than in the fall (Fig. 1-4)

I regressed d_b on d_e for each individual to determine if thermal quality influenced thermoregulatory effort in milksnakes on a daily basis. There was no significant difference between males and females ($t_{28} = 0.927$, $d = 0.17$, $p = 0.36$) and, therefore, I pooled the slopes for both sexes. The mean slope was 0.69 ± 0.09 and was significantly less than 1 ($t_{39} = 3.39$, $p = 0.001$) indicating that thermoregulatory investment decreases as thermal quality increases (Fig. 1-5).

Habitat Use and Behaviour

I calculated the difference in percentage of open habitat for snake and random locations and used a two-way ANOVA, with sex as a control variable, to determine if milksnakes used open habitats proportionally more in any of the three seasons. Milksnakes used open habitats to the same extent in all three seasons ($F_{2,72} = 0.0093$, $R^2 = 0$, $P = 0.99$) which was contrary to my prediction that milksnakes would select higher thermal quality habitat in the spring and fall (Fig. 1-6). There was no significant effect of sex ($F_{1,72} = 0.014$,

$R^2 = 0.01$, $P = 0.91$). Snake locations were more often in the open than random locations (paired t-test with data for both sexes and the 3 seasons: $t_{72} = 6.47$, $d = 27.77$, $p < 0.0001$). This demonstrated that milksnakes were using open habitats proportionally more than they were available, but that the difference did not vary across the seasons as I had predicted.

I analyzed the behaviour of the snakes to determine if it varied across seasons. Because these values are categorical, it was impossible to average across individuals and some pseudo-replication resulted, which may effect the level of significance. There was no difference in behaviour between males and females ($\chi^2_2 = 4.36$, $p = 0.11$) and, thus, I pooled the data for both sexes and all behaviours. Milksnake behaviour did vary significantly across seasons ($\chi^2_4 = 81.95$, $p = 0.0001$) (Fig. 1-7). Milksnakes were seen basking/resting most in the spring ($\chi^2_2 = 62.92$, $df = 2$, $p < 0.001$), traveling most in the summer ($\chi^2_2 = 13.05$, $df = 2$, $p = 0.001$), and were concealed most often in the summer and fall ($\chi^2_2 = 26.3$, $df = 2$, $p < 0.001$) (Fig. 1-7).

Milksnakes invested more in thermoregulation in the spring than in the summer and fall. They did not, however, alter their habitat use to achieve higher T_b , instead they were basking more often, which resulted in higher T_b s. Milksnakes were investing more into thermoregulation in forests (lower thermal quality) than in open habitats, but this was not reflected in a significant change in behaviour.

Movements

I calculated distances moved per day and then performed a two-way ANOVA to determine if movement rates varied between sexes or between seasons. The distance traveled was log transformed to meet the assumptions of ANOVA. There were no significant differences between the sexes ($F_{1,71} = 1.05$, $R^2 = 0.16$, $P = 0.31$), but there were seasonal

differences ($F_{1,72} = 6.06$, $R^2 = 0.16$, $P = 0.0038$). Tukey-Kramer HSD tests revealed that milksnakes moved more in the summer than in the spring and fall (Fig. 1-8). Males often move more than females during the mating season (Brito 2003). Milksnakes in Ontario mate in late May or early June (Row, personal observation) and, thus, males might move more in the spring. The data suggest that it could be the case (Fig. 1-8), but the interaction between sex and season was not significant ($F_{1,72} = 1.35$, $R^2 = 0.16$, $P = 0.26$).

Prey Density

To determine if prey density influenced habitat use I used a 2 way ANOVA to determine if prey density varied with type of habitat or season. I log transformed the data in an effort to meet the assumptions of ANOVA, but normality could not be achieved. ANOVA, however, is robust to departures from normality and none of the factors were close to the significance threshold. There was a significant interaction between season and habitat ($F_{2,147} = 8.09$, $R^2 = 0.15$, $P = 0.0005$). Prey density was higher in open habitats in the spring ($Z_{41} = -2.02$, $p = 0.04$), but higher in forested habitats in the summer ($Z_{80} = -3.00$, $p = 0.0027$) and fall ($Z_{21} = 2.83$, $p = 0.0045$) (Fig. 1-9). If prey density was affecting habitat use, then milksnakes should have been in open habitats more in the spring and in forested habitats more in the summer and fall. This was not the case, however, and milksnakes had a consistent preference for open habitats in all three seasons.

Discussion

Investment in thermoregulation varies with thermal quality and recent evidence suggests that ectotherms invest more in thermoregulation when the thermal quality is low (Blouin-Demers and Weatherhead 2001b, Blouin-Demers and Nadeau 2005). This is contrary to the central prediction of the cost-benefit model of thermoregulation (Huey and Slatkin

1976) that predicts reptiles should invest less in thermoregulation when the costs are high. Milksnakes had a higher effectiveness of thermoregulation ($d_e - d_b$) in the spring than in the summer. Thus, consistent with my prediction, snakes were investing more in thermoregulation in the spring when the thermal quality was low. In the fall, however, milksnakes were investing significantly less in thermoregulation than in the spring and the summer. The thermal qualities of all habitats were similar in the spring and fall and, thus, I expected that snakes should adopt similar thermoregulatory strategies. This difference, however, could be attributed to ecological factors other than temperature. During hibernation reptiles have a temperature-induced torpor, in which they have little or no metabolic activity (Gregory 1982). Because of this reduced metabolism, reptiles cannot digest food during hibernation and, therefore, must fast prior to hibernation (Gregory 1982). During this fasting period milksnakes do not need to elevate T_b to digest, forage or find mates and, therefore, should not engage in basking because it is costly in time, energy, and risk of predation. Milksnakes in the study area went into hibernation in late September or early October and, therefore, the pre-hibernation fasting period could have encompassed the majority of the fall, leading to the reduced effort in thermoregulation.

In the spring and fall, milksnakes invested more in thermoregulation in forested habitats than in open habitats. Because thermal quality was lower in forests, this further supported the prediction that milksnakes should invest more in thermoregulation when the thermal quality is low. In the summer, investment in thermoregulation by milksnakes was the same in open and forested habitats, even though open habitats had a higher thermal quality. Thermal quality in the summer was higher in both habitats, but the difference in thermal

quality between open and forested habitats was less than in the spring and fall, most likely leading to similar investment in both habitats.

Milksnakes also responded to daily variation in thermal quality and invested more in thermoregulation when thermal quality was low. This reinforces the tight link between thermal quality and investment in thermoregulation and provides more evidence contradicting the central prediction of the cost benefit model of thermoregulation. In temperate climates, thermal quality is low and, therefore, there are large costs in terms of energy, time, and exposure to predators that an individual incurs to maintain T_b within T_{set} . In temperate climates, however, there are also disadvantages to thermoconformity because low temperature will lead to reduced organismal performance. The habitat in Ontario is thermally challenging and, in milksnakes, it appears that the disadvantages associated with thermoconformity when thermal quality is low are more important in influencing investment in thermoregulation than the costs incurred for thermoregulation. These results support growing evidence that the central prediction of the cost benefit model of thermoregulation related to thermal quality does not apply broadly (Blouin-Demers and Weatherhead 2001b, Blouin-Demers and Nadeau 2005). The model seems to be more applicable in thermally benign habitats where the disadvantages of thermconformity are lower (Lee 1980, Hertz and Huey 1981).

Habitat Use and Behaviour

Milksnakes had a strong preference for open habitats over forested habitats. This preference was equally strong for both sexes and in all three seasons. I believe that the best explanation for this preference is that it facilitates behavioural thermoregulation.

The main food source of adult milksnakes are small mammals (Williams 1988), which were caught more often in forests than in open habitats, in all seasons except the spring. Thus, if milksnakes were selecting habitats based primarily on mammalian prey abundance, they should have a preference for forests in summer and fall. Milksnakes, however, had the same preference for open habitats in all three seasons and, therefore, milksnakes were not preferentially using open habitats for foraging.

Another possible explanation for the preference for open habitats could be predator avoidance. I do not have data on the abundance of milksnake predators by habitat and cannot assess this possibility formally. Because the proportions of risky behaviours (traveling, basking) were the same in all habitats, however, it suggests that milksnakes were not trying to avoid predators more in one habitat than in another.

Finally, ectotherms must obtain heat directly from the environment and, therefore, alter their T_b through habitat selection and behaviour. In all three seasons, open habitats provided a higher percentage of T_e 's that were within T_{set} . Similarly, open habitats facilitate thermoregulation by offering a wider range of T_e s and by providing the opportunity to shuttle between basking and shelter, which should allow the snakes to maintain T_b s with T_{set} for 7 hours per day. Maintaining T_b within T_{set} was never possible in forests. These observations point to a relationship between preference for open habitats and thermoregulation and, thus, support my prediction that habitat use should be tightly linked with thermoregulation. This link should be especially tight in thermally challenging environments where the disadvantages of thermoconformity are much higher than in thermally benign environments.

Because of the link between habitat selection and thermoregulation, I expected milksnakes to select higher thermal quality habitats in the spring and fall when thermal

quality was low. Contrary to my prediction, milksnakes did not alter their preference for open habitats through the season. My classification of habitats was coarse and, although I am confident in the results, I realize that habitat selection can occur on much finer scales. Habitat features such as size of rock have been shown to have a role in the selection of retreat sites by snakes (Huey and Kingsolver 1989, Webb and Shine 1998). Habitat data collected on a finer scale might provide better insight and demonstrate a stronger link between seasonal habitat selection and thermoregulation.

Milksnakes shifted their behaviour throughout the seasons to increase thermoregulation. Instead of using open habitats more often, milksnakes achieved higher T_b s by spending more time basking and less time concealed. By exposing themselves a higher percentage of time in the spring milksnakes were able to maintain T_b s the same as in the summer despite the summer having a higher thermal quality. In the fall, milksnakes had significantly lower T_b s than in the spring and summer, despite the possibility of obtaining T_b s similar to the spring. The percentage of time basking in the fall, however, was the lowest in any of the three seasons and, as a result, in the fall the T_b s and effectiveness of thermoregulation was the lowest for any of the seasons.

Milksnakes also had higher rates of movement in the summer than in the spring and fall. This is most likely a consequence of the high thermal quality and reduced investment in thermoregulation in the summer. In the spring, milksnakes invested more in thermoregulation and, therefore, had to spend more time basking, which does not require long movements. In the summer, milksnakes were expending less effort in thermoregulation and had more time to forage, which requires longer movements. Milksnakes mate in late May and June and, thus, the increased movement in the summer could not be related to mating. No gravid females

were included in the analysis and, thus, the increased movements in the summer are not a consequence of nest searching. In the fall, however, milksnakes were expending the least amount of effort into thermoregulation, but still had lower movement rates than in the summer. Again this is likely related to the fasting period before hibernation (Gregory 1982) where milksnakes would not be foraging and, therefore, should not perform risky behaviours such as traveling that expose them to predators.

Blouin-Demers and Nadeau (2005) found with lizards that investment in thermoregulation decreased when thermal quality increased, which was opposite to the central prediction of the cost-benefit model of thermoregulation. The explanation they presented was that the physiological consequences of thermoconformity are higher when the thermal quality is low. This was a comparative study at a global scale, however, and because the cost-benefit model was designed for single species they suggested that more rigorous tests of the model should be conducted. Thermal quality was higher in the summer than in the spring and in open habitats than in the forests. Milksnakes invested more in thermoregulation in the spring and in forested habitats. Similarly milksnakes invested more in thermoregulation when thermal quality was low on a daily scale. These results demonstrate that milksnakes invest more in thermoregulation when thermal quality is low supporting their conclusions. More tests of the model in varying environments are necessary to determine if these results are consistent across species and in different environments. Tests of the same species in different environments (thermally benign versus thermally challenging) might provide better insight into the costs of thermoregulation in different environments. Furthermore, as suggested by Bloiun-Demers and Nadeau (2005), laboratory

experiments are needed to decouple the costs and benefits of thermoregulation to better assess their influence on thermoregulation.

Milksnakes selected a high percentage of open habitat to facilitate behavioural thermoregulation. Contrary to my predictions, however, there was no difference in habitat use when investment in thermoregulation was higher. Instead milksnakes were seen to shift their behaviour. It is unlikely that changes in thermoregulatory investment would not be coupled with changes in habitat use. My classification of habitat use was coarse and habitat classified at a finer scale would provide a more complete picture of the effect of thermoregulation on habitat use.

Table 1-1. Multiple regression equations used to predict model snake temperatures in different habitats available to eastern milksnakes in Ontario.

Habitat	R	Equation	F	df	P
Field	0.92	$1.2T + 38.00Rd + 0.23W + 0.0025S + 0.69R - 8.10$	44000	5,954	<0.001
Forest under leaves	0.91	$0.71T + 0.082Rd + 0.062W + 2.0$	3300	3,956	<0.001
Forest under log	0.77	$0.44T - 3.2Rd + 0.045R - 0.064W + 7.1$	790	4,955	<0.001
Forest under rock	0.79	$0.52T - 2.2Rd + 6.9$	1800	2,957	<0.001
Forest*	0.91	$0.12T + 0.56Rd + 0.013W + 1.84$	3300	3,956	<0.001
Rocky Outcrop on Grass	0.94	$0.99T + 33Rd + 0.14W + 0.31R - 3.59$	3600	4,955	<0.001
Rocky Outcrop under rock	0.87	$0.99T - 0.75Rd - 0.074W + 1.3$	2210	3,956	<0.001
Rocky Outcrop under log	0.89	$0.72T - 1.23Rd - 0.022W + 4.7$	3200	3,956	<0.001
Rocky Outcrop on rock	0.96	$1.0T + 22Rd - 0.087W - 0.087$	7800	3,956	<0.001
Field under grass	0.81	$0.82T + 8.7Rd + 0.11W + 0.17R + 0.66S - 12$	820	5,978	<0.001

The variables entered as predictors were air temperature (T) in °C, solar radiation (Rd) in kW/m², wind speed (W) in m/s, soil temperature (S) in °C and rainfall (R) in mm/day. *Root square transformed the temperatures before regression.

Table 1-2. Mean $\pm$ SE, maximum, and minimum operative environmental temperatures (T_e) recorded in the four main habitats available to eastern milksnakes in Ontario. Mean $\pm$ SE, maximum and minimum and percent equal to zero deviations of operative environmental temperatures (d_e) from T_{set} of eastern milksnakes.

Spring						
Habitat	T_e			d_e		
	Mean	Max	Min	Mean	Max	% = 0
Open-No shelter	31.66 $\pm$ 0.36	57.23	6.26	8.80 $\pm$ 0.18	27.57	15
Open-Shelter	20.15 $\pm$ 0.18	39.45	7.81	9.18 $\pm$ 0.15	19.18	3
Forest-No shelter	19.91 $\pm$ 0.15	45.18	7.00	9.46 $\pm$ 0.13	20.02	9
Forest-Shelter	15.08 $\pm$ 0.07	26.31	9.59	13.92 $\pm$ 0.07	19.66	0
Open	25.79 $\pm$ 0.24	57.23	6.26	9.13 $\pm$ 0.11	27.57	23
Forested	17.61 $\pm$ 0.12	39.45	7.34	11.55 $\pm$ 0.11	17.41	3
Summer						
Open-No shelter	34.35 $\pm$ 0.30	56.03	15.48	8.19 $\pm$ 0.18	26.36	18
Open-Shelter	24.04 $\pm$ 0.14	37.45	12.48	5.50 $\pm$ 0.12	14.15	9
Forest-No shelter	23.89 $\pm$ 0.12	42.14	13.71	6.06 $\pm$ 0.07	13.28	15
Forest-Shelter	17.28 $\pm$ 0.05	25.30	11.48	11.71 $\pm$ 0.05	15.52	0
Open	29.12 $\pm$ 0.19	56.03	13.71	7.13 $\pm$ 0.09	26.36	31
Forested	20.66 $\pm$ 0.10	37.45	11.48	8.61 $\pm$ 0.08	13.89	9
Fall						
Open-No shelter	28.13 $\pm$ 0.31	53.53	2.21	8.03 $\pm$ 0.16	24.78	16
Open-Shelter	20.03 $\pm$ 0.17	33.79	6.17	9.09 $\pm$ 0.16	20.82	2
Forest-No shelter	20.54 $\pm$ 0.14	40.25	1.23	8.77 $\pm$ 0.13	25.76	8
Forest-Shelter	15.53 $\pm$ 0.08	23.48	4.36	13.47 $\pm$ 0.08	22.64	0
Open	23.34 $\pm$ 0.22	53.53	1.23	8.40 $\pm$ 0.12	25.76	22
Forested	17.78 $\pm$ 0.12	33.79	4.36	11.28 $\pm$ 0.12	22.64	2

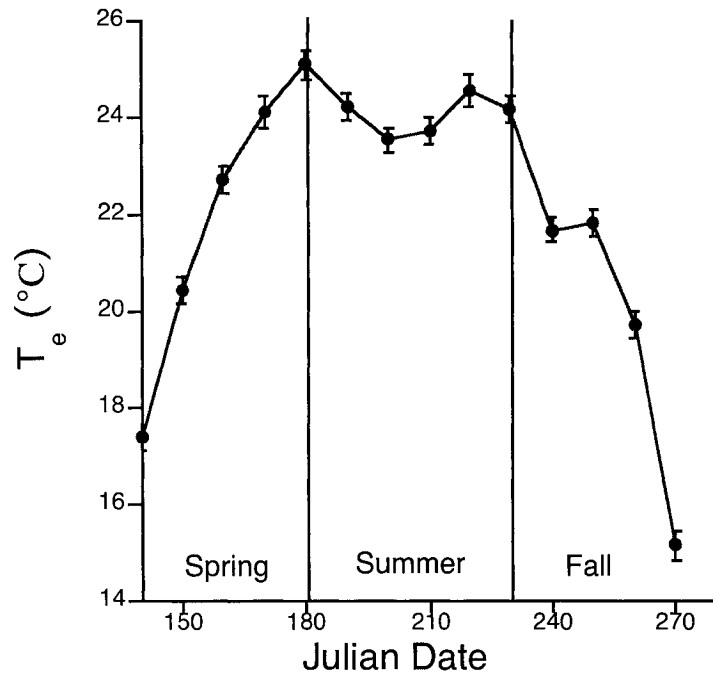


Fig. 1-1: Mean operative environmental temperatures ($T_e \pm 1$ SE) for all habitats in 2003 and 2004 are lower in the spring and fall than in the summer. The active season was divided into three seasons to maximize differences in temperature, while keeping sample sizes similar.

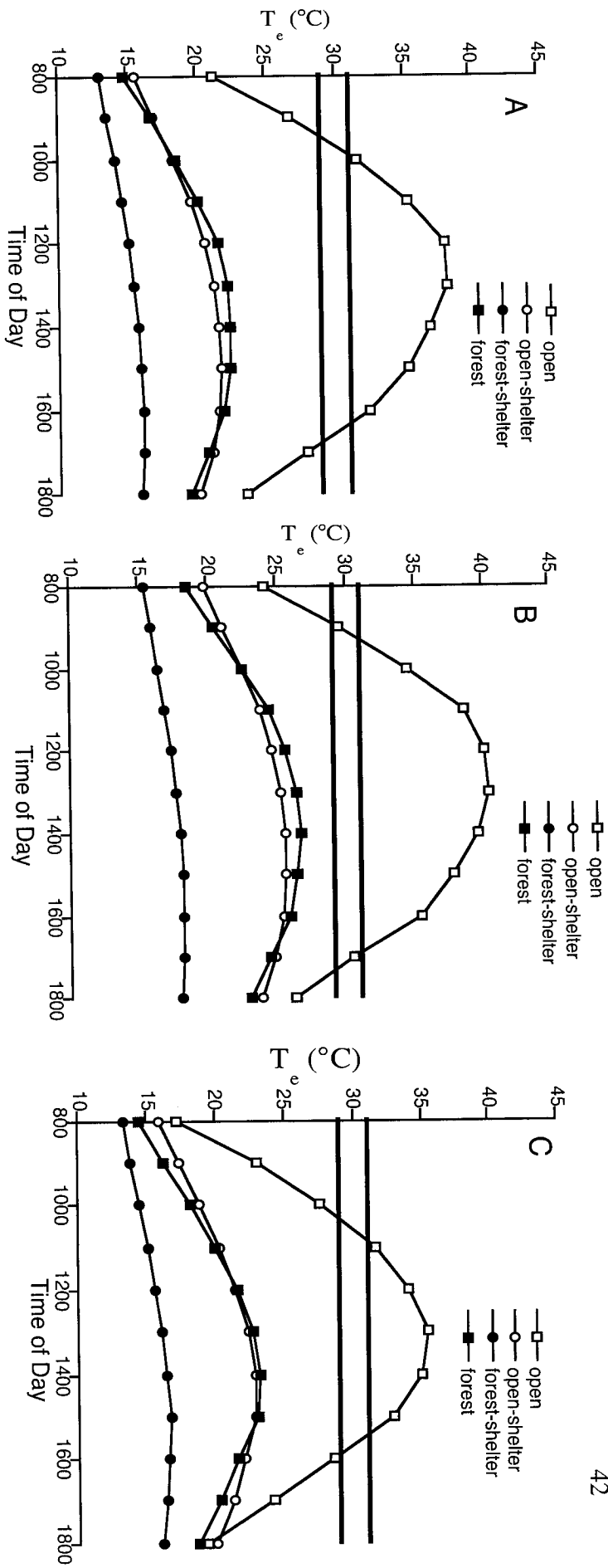


Fig. 1-2: Mean operative environmental temperatures (T_e) for each hour of the day in four different habitats in the A) spring, B) summer, and C) fall in Ontario. The range of preferred body temperature (T_{set}) is represented by the solid black lines.

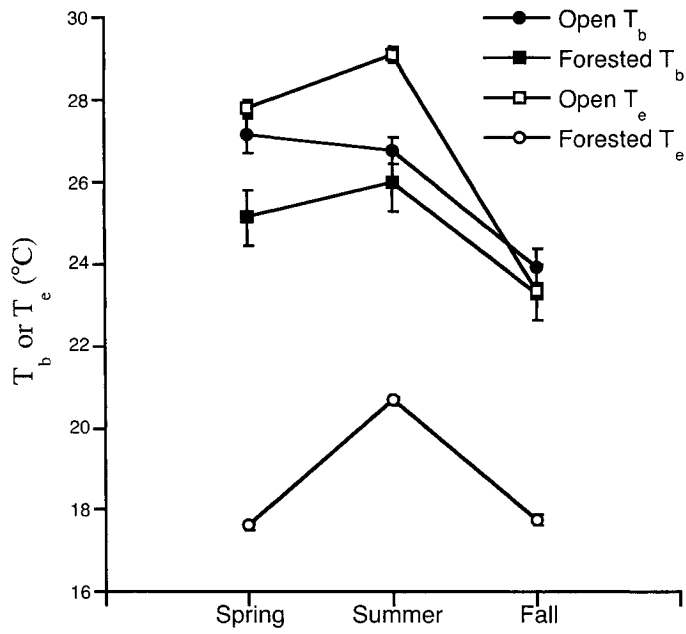


Fig. 1-3: Weighted least square mean (weighted by sample size and corrected for sex) body temperature ($T_b \pm 1$ SE) in eastern milksnakes in different habitats and seasons. Operative environmental temperatures ($T_e \pm 1$ SE) were higher in open habitats than forests and in the summer than in the spring and fall in Ontario in 2003 and 2004.

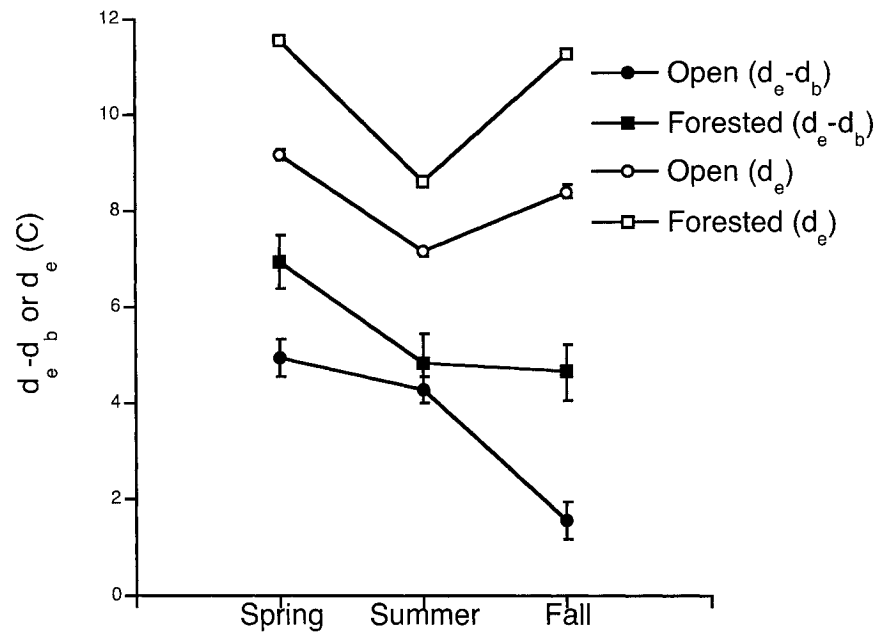


Fig. 1-4: Weighted least square mean (weighted by sample size and corrected for sex) thermoregulatory effectiveness index ($d_e - d_b \pm 1$ SE) showing a higher investment in thermoregulation in forested habitats than open habitats in all three seasons. Calculated by the opportunities offered in each separate habitat during the day (8:00-16:00) in Ontario. Mean deviations of operative environmental temperatures from T_{set} in eastern milksnakes showing a higher thermal quality in open habitats in Ontario in three different seasons.

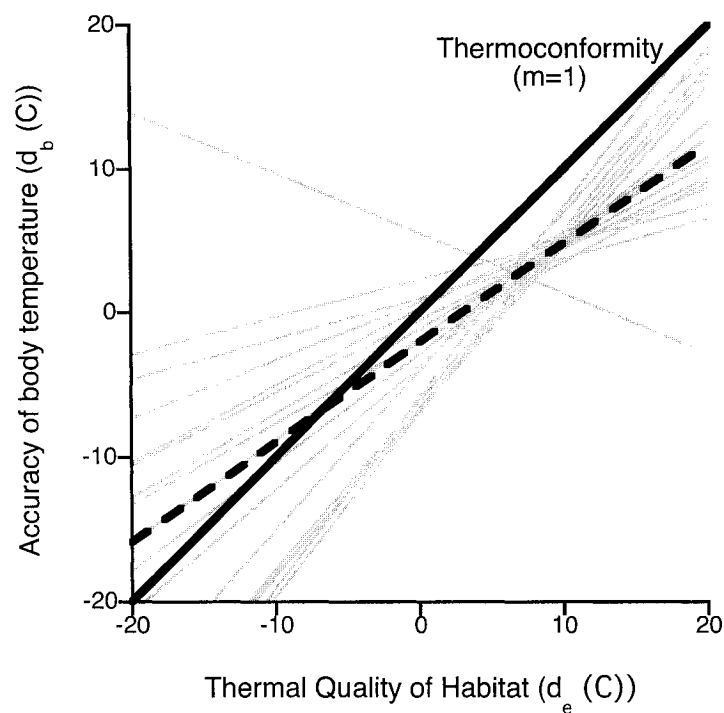


Fig. 1-5: Eastern milksnakes thermoregulate more effectively (high $d_e - d_b$) when the thermal quality of the habitat (d_e) is low during the day (800-1600) in Ontario. This is indicated by the mean slope (dashed line) being significantly less than 1 (dark line) (thermoconformity). The grey lines represent the slopes for individual milksnakes.

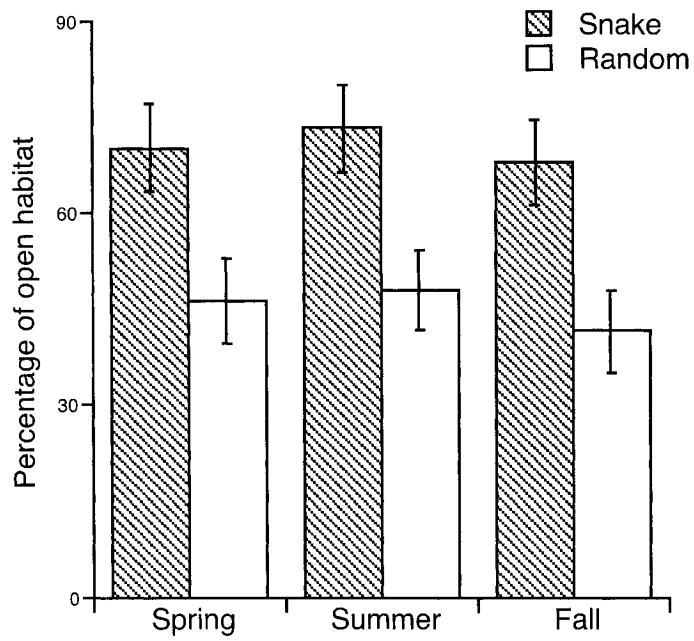


Fig. 1-6: Mean percentage of open habitat ($\% \pm 1 \text{ SE}$) is consistently higher in eastern milksnake locations than in random locations across all three seasons in Ontario in 2003 and 2004.

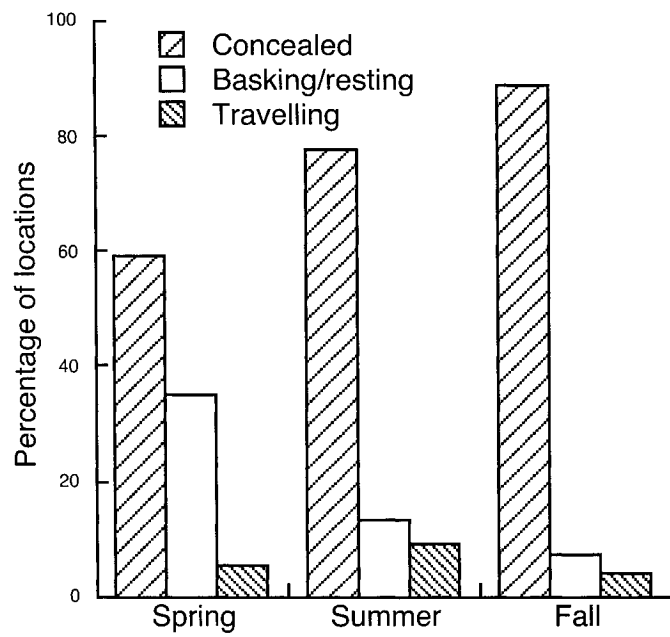


Fig. 1-7: A higher percentage of eastern milksnakes were concealed and a lower percentage were basking when located in the summer and fall than the spring in Ontario in 2003 and 2004.

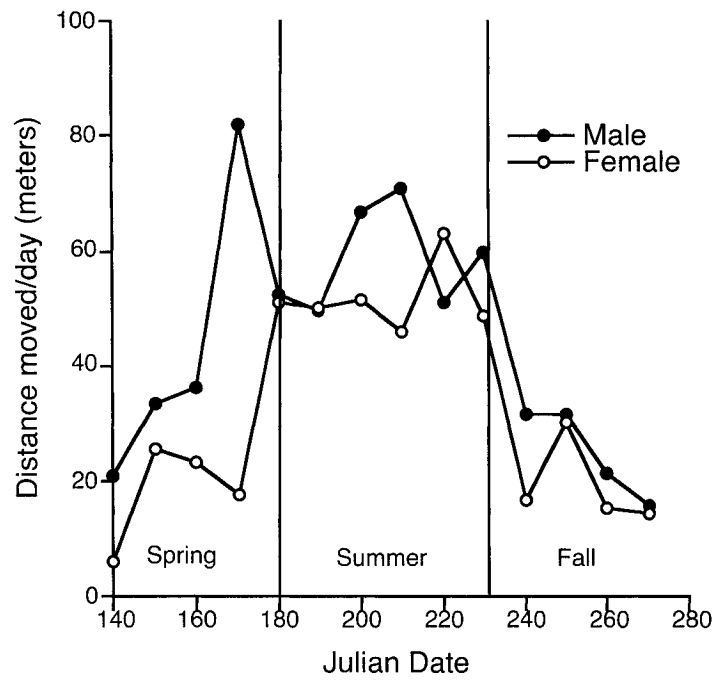


Fig. 1-8: Eastern milksnakes have a higher mean rate of movement (distance traveled per day) in the summer than in the spring and fall in Ontario in 2003 and 2004.

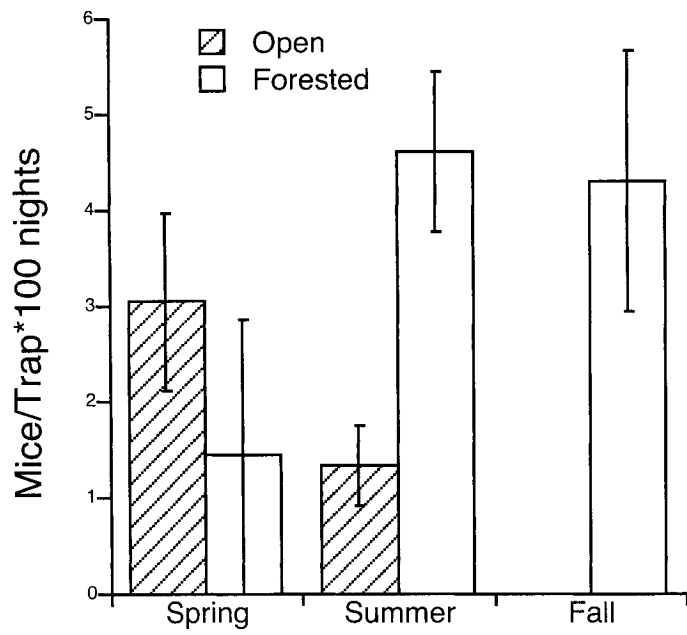


Fig. 1-9: Mean prey density (mice/trap * 100 nights ± 1 SE) of eastern milksnakes is higher in forested habitats in the summer and fall but higher in open habitats in the spring in Ontario.

CHAPTER 2

Habitat selection at multiple spatial scales: influences of thermal quality and implications for the use of remote sensing at very fine scales

Introduction

An organism has many choices when selecting a habitat, most of which can occur on different spatial scales (Orians and Wittenberger 1991). Johnson (1980) divided habitat selection into distinct scales: first order selection is the geographical range of the species, second order selection is the selection of a home range within that geographical range, and third order selection is the selection of specific locations within the home range. Selection of habitat at these different spatial scales can be viewed as a hierarchical process and, in many organisms, it has been shown that it is important to consider selection at more than one scale (Orians and Wittenberger 1991, Odom et al. 2001, Luck 2002, Stephens et al. 2003). Many selection pressures and limiting factors may be scale dependent, thus leading to different patterns of selection at different scales (Rettie and Messier 2000). By limiting the study of habitat selection to a single spatial scale, important factors influencing habitat selection can be missed.

Macro and microhabitat selection have often been equated with second and third order selection (Johnson 1980), respectively, which have been used in describing the ecology of numerous species (Blouin-Demers and Weatherhead 2001a, Luck 2002, Boyce et al. 2003). Although there have been many studies on microhabitat selection, few studies have investigated macrohabitat selection or selection at multiple spatial scales. Habitat selection studies on birds and mammals are often conducted at more than one scale, but this is rarely the case in reptiles. Compton et al. (2002) examined habitat selection in wood turtles at two spatial scales and found selection at both scales. At the watershed level, wood turtles selected activity areas that were partially forested, but within their activity areas turtles selected areas with low canopy cover. This was believed to represent a trade-off between feeding and

thermoregulation and highlights the importance of scale when determining habitat selection in reptiles.

In reptiles, it has been shown that distribution of prey (Madsen and Shine 1996) and hibernation sites (Reinert and Kodrich 1982, Reinert 1993) can affect habitat selection. Reproductive state (Reinert 1984, 1993, Blouin-Demers and Weatherhead 2001a) can also alter thermoregulatory behaviour and, thus, habitat selection. Because of the importance of thermoregulation to fitness in ectotherms (Christian and Tracy 1981, Huey and Kingsolver 1989), however, temperature is believed to be the most important factor influencing habitat selection in terrestrial squamates (Reinert 1993). Thermoregulation is particularly important to reptiles in temperate climates because the lack of thermoregulation results in T_b that drastically decreases performance when the environment is thermally challenging (Blouin-Demers and Weatherhead 2001b). I conducted this study on eastern milksnakes in a temperate climate at the northern extreme of their range. My primary goal was to determine if habitat selection in milksnakes is affected by thermal quality at multiple spatial scales. I predicted that milksnakes should select habitats of high thermal quality at both the micro and macrohabitat scale. In my study area, habitats of high thermal quality are open habitats (e.g., rocky outcrops, fields) and edges between forest and open habitats (Chapter 1, Blouin-Demers and Weatherhead 2001) that allow the snakes access to solar radiation for basking.

Remote sensing has become a popular tool to classify, monitor, and identify habitats of organisms for conservation purposes. Remote sensing is faster and cheaper than field surveys for broad scale habitat classification (Fuller et al. 1989). The identification of the habitat used by species of conservation concern has important political and regulatory ramifications because of the protection afforded to the habitat of threatened species in many

jurisdictions (Herr and Queen 1993, Reunanen et al. 2000, Odom et al. 2001, Osborne et al. 2001). As remote sensing technology is advancing and becoming more widely used, more researchers attempt to apply it to a finer scale. Despite the growing popularity of remote sensing approaches, very little work has been done to test the effectiveness and the accuracy of these methods in the context of habitat selection.

In this study, I used high resolution satellite imagery to investigate habitat selection at two spatial scales: the home range scale and the location scale. I also used traditional field habitat characterization to investigate habitat selection at the location scale. The secondary objective of this chapter is to evaluate the ability of remote sensing approaches to document habitat selection by milksnakes. I compared the results of the two parallel approaches: the remote sensing approach and the field surveys. This will help determine if remote sensing and GIS tools are useful in conjunction with, or instead of, field surveys to identify habitat selection patterns on a fine scale.

Methods

Study Area and Study Species

I conducted this study at the Queen's University Biology Station, 150 km south of Ottawa, Ontario (44°34' N, 76°19' W) from August 2002 to November 2004. The study area was approximately 8 by 3 km and was characterized by rolling terrain with numerous granite rocky outcrops, small lakes, and marshes. The study area is mainly composed of second growth deciduous forest with some coniferous trees present in drier areas. The abandonment of marginal farmland has also resulted in old fields and scrub habitats being common. Some of the more productive fields are still hayed. Eastern milksnakes (*Lampropeltis triangulum*)

are medium-sized (to 92 cm SVL and 210 g) snakes that feed mostly on small mammals. Milksnakes exist at low density, but occur throughout the study area.

Radio-telemetry

I captured experimental animals at black ratsnake hibernacula monitored during spring emergence or opportunistically throughout the active season. Hibernacula were monitored by surrounding them with plastic fencing fitted with funnel traps (Blouin-Demers et al. 2000a). After capture, I probed the cloaca of the snakes for the presence of hemipenes to determine sex, measured their snout-vent length (SVL) with a flexible measuring tape, weighed them with an electronic scale, and marked them by injecting a passive integrated transponder (PIT tag) subcutaneously.

I 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). The transmitter weighted 5.5 g and never exceeded 5% of the snakes' mass. I aimed at implanting an equal number of females and males, but this was not possible because I captured fewer females than males and because females are smaller than males and, therefore, fewer females were large enough to implant. Transmitters were implanted under sterile conditions and isoflurane anaesthesia (Blouin-Demers et al. 2000b, Weatherhead and Blouin-Demers 2004).

I released the snakes at their point of capture one day after implantation and located them every 2-3 days using a telemetry receiver (Wildlife Materials, Murphysboro, IL, Communications Specialists, Orange, CA, or AVM instruments, Colfax, CA) and a directional Yagi antenna. At each location, I recorded the UTM coordinates (NAD83 datum) with a GPSmap76 (Garmin International Inc., Olathe, KS), the position of the snake, and the

behaviour of the snake. I used the UTM coordinates to calculate distances moved between locations.

Home Ranges

Minimum convex polygon (MCP) is the most commonly used home range estimator (Powell 2000). It simply places 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 some 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 and 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 and 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, using simulations 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) and has lead to the conclusion that kernels are more suited to analysis of use rather than to the estimation of home range size (Harris et al. 1990). Methods for choosing the smoothing parameter have been developed. Least squared cross validation (LSCV) has been shown to be the most accurate for most

situations and is the most widely used (Worton 1989, Seaman and Powell 1996, Stevens and Barry 2002, Morzillo et al. 2003). This method attempts to choose the smoothing parameter that has the lowest error (Seaman and Powell 1996). 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 and Powell 1996). Milksnakes used the same location multiple times and, thus, locations are autocorrelated. Subsampling is suggested when data are autocorrelated (Worton 1987). Milksnakes were located on average every 2-3 days, and the distances they cover when they move indicate that this period is ample time to traverse an entire home range and, therefore, I believe it was the snake's choice to stay at a given location and not an impossibility to reach a new location during the time interval between telemetry locations. Subsampling may eliminate autocorrelation in this case, but it would reduce the biological significance of the home range estimate (De Solla et al. 1999).

Instead of subsampling, I 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 of the kernel. Most 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 1 is extremely unlikely, the 95% kernel is most often used to calculate the total home range of an animal (Powell 2000). Therefore, for each individual I used the MCP as the area of the home range and adjusted the smoothing parameter until the area of the 95% kernel equaled the area of the MCP.

Land Cover Classification

I derived land cover classification from high definition IKONOS imagery (Space Imaging, Thorton, CO) with a pixel size of 4 m. I used GRASS 5.0 (GRASS Development Team 2003) to georeference the imagery and to perform a supervised sequential maximum a posteriori (SMAP) classification (Schowengerdt 1997). I georeferenced the image with 42 easily identifiable locations spread evenly throughout the study area and then rectified the image to fit the control points with a cubic transformation (i.rectify command). I obtained a root mean squared error of ca. 5 meters. I classified the image (i.smap command) into 5 habitat classes: field, open water, forest, marsh and rocky outcrop. I used approximately 20 training sites per habitat type. I 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 sampled the range of variation present within each habitat type. After the classification was complete, I determined classification accuracy. I generated approximately 20 random locations in each habitat type of the classified image and I traveled to those points with a GPS unit to classify the habitat on the ground. The average classification accuracy for all habitats was 90 % and marshes had the lowest classification accuracy (77 %) of all the habitats (Table 2-1).

Macrohabitat Selection

To determine which habitats milksnakes selected at the home range scale, I needed to compare the habitat use of the individual to the habitat that was available. To quantify habitat use, I determined the percentage of field (FLD), rocky outcrop (RO), marsh (MAR) and forest (FOR) in the 95%, 75% and 50% kernels. Open water was excluded from the home range and the analysis. Habitat was not homogeneous throughout the study area and,

therefore, I did not want to use the composition of the study area as the available habitat for all individuals. Instead, I used the composition of a circle centered on the hibernacula or the first location (if the hibernacula was not known) with a radius equal to the length of the home range. I then compared the percentages of use to the percentage of availability of each habitat type. The proportions of the 4 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 $y_i = \text{LN}(x_i/x_j)$ 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. To determine if habitat use was non-random and differed between the sexes, I log-ratio transformed all the percentages of use of habitats, using %MAR as the denominator. I then used a MANOVA to determine if habitat use differed from available habitat.

If habitat use was non-random with respect to availability, I assigned ranks to the habitats based on use to available log ratios (Aebischer et al. 1993). Following the methods of Aebischer (1993), I created a matrix to establish the rank of each habitat with respect to all other habitats (Table 2-2).

I defined an edge as the transition from a two-dimensional habitat (field, rocky outcrop, marsh) to a three-dimensional habitat (forest). Snakes are often attracted to edges because they facilitate thermoregulation by offering simultaneous access to sun and shade (Blouin-Demers and Weatherhead 2001a). To determine if milksnakes preferred edges at the home range scale, I calculated the length of edge per hectare in each kernel density estimate. I did the same for the circle of habitat available to each individual. For each individual, I calculated the difference in edge length between use and availability. I used t-tests to

determine if the difference in length of edge per hectare between use and availability was significantly different from zero and was significantly different between the sexes.

Microhabitat Characterization

Each time I located a snake, I recorded the habitat type (field, rocky outcrop, forest, marsh). Patches of habitat less than 15 X 15m were ignored.

I also conducted a more detailed habitat characterization at all locations used by radio-implanted milksnakes, except when they were in buildings or human debris because these locations could not be characterized adequately with my sampling scheme. I also did not characterize locations that were < 10 m from a characterized location because 10 m was the largest sampling radius used during characterization. I characterized the habitat approximately one week after the snake had moved to a new location to ensure that the snake was not disturbed while minimizing structural changes occurring between use and characterization. Locations that were used more than once were included only once in the analysis.

To determine if snakes were using habitat non-randomly, I had to quantify available habitat by repeating the same characterization at 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, I measured 24 structural variables in circular plots of different radii (Table 2-3). I chose sampling radii based on the smallest possible radius where variation was present and measurable. To evaluate ground cover and canopy closure, I used a

sighting tube (50 x 2.5 cm piece of pipe) with a cross wire at one end (Winkworth and Goodall 1962). I aimed the tube randomly at the ground 50 times within the 1 m radius and recorded the number of times each different cover type was sighted in the cross hairs. Similarly, to measure canopy closure I lay on the ground (to have the snake's perspective) and aimed the tube randomly 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, the number of hits were multiplied by two to obtain a percentage.

Microhabitat Selection Based on Land Cover

From the general habitat types used, I determined if use was non-random. Then, I ranked the habitat types in order of preference. I used log ratios and the same method as in the macrohabitat analysis to evaluate habitat selection within the home range. Habitat use was quantified by taking the percentage of locations within each habitat type. In the macrohabitat analysis, I determined if milksnakes selected their home range randomly by comparing the composition of the home range to the composition of the surrounding habitat. Here I determined if milksnakes selected locations within the home range randomly and, therefore, I used the composition of the 95 % kernel as the habitat available to each individual.

Microhabitat Selection Based on Habitat Characterization

MANOVA followed by a discriminant function analysis or logistic regression are used most commonly in habitat selection studies where habitat availability is quantified based on sampling at random locations. For both methods, used locations are compared to all random locations; assuming that conditions are the same through time and throughout the study area and that each individual has access to all random locations.

To analyze microhabitat selection based on habitat characterization, I used matched-pairs logistic regression. Logistic regression is more robust than MANOVA and discriminant function because it does not require multivariate normality (North and Reynolds 1996). 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. Milksnakes are cryptic and, thus, it was impossible to completely rule out the potential use at the random locations by the snakes. When use is rare, however, it is acceptable to use a case-control design because it is then expected that the random locations will be made up of almost exclusively unused locations (Keating and Cherry 2004). Milksnakes exist at low density in the study area and no milksnakes were ever seen at random locations and, therefore, it seems valid to assume that milksnake locations are rare.

In the matched-pairs logistic regression, I subtracted the values for all the habitat variables (Table 2-3) at each random location from the values at each paired snake location. I then used standard logistic regression with the constant term excluded to fit a response vector of all 1's. The estimated coefficients are interpreted the same way as with standard logistic regression. For a coefficient β_i , an n -unit increase in the habitat variable corresponds to an $e^{n\beta_i}$ increase in the odds ratio. Because the presence of a milksnake is a low probability event, the odds ratio estimates the relative risk, which is the ratio of the probability of x (the presence of a milksnake) given A (variables measured at milksnake location) to the probability of x given B (variables measured at the random location) (Breslow and Day 1980). Also, because in the paired design, the variables are differences in values between snake and random observations, the model is interpreted as differences in the habitat and not as absolute measured values.

An assumption of logistic regression is that each observation is sampled at random. This is hard to achieve in radio-telemetry studies where many locations are obtained from few individuals. In this study, I 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 I had many variables in the model, it would have been impossible to fit individual models for snakes for which I had few locations. Instead, I chose to fit models separately for males and females. All individuals were similar in size and, therefore, roughly the same age. Since I did not include any gravid females in the analysis, I did not expect a difference between males and females. Because there is a slight sexual dimorphism (males being larger), however, I analyzed the sexes separately.

Based on preliminary univariate and multivariate analyses, I selected candidate models. I chose the candidate models conservatively and included variables most likely to account for the differences between snake and random locations. Single members of groups of highly correlated variables were included in the candidate models to avoid shadowing of important variables. Candidate models were compared against each other using Akaike's Information Criterion (AIC) (Burnham and Anderson 1998). The linearity of the final models was tested using design variables based on the quartiles of each variable (Hosmer and Lemeshow 2000).

Comparison of Remote Sensing and Traditional Approaches

To quantify habitat selection with remote sensing, I used buffers around each location to limit bias and to control for error in the GPS and the satellite imagery (Rettie and McLoughlin 1999). Rettie and McLoughlin (1999) demonstrated that the choice of buffer

width can have an effect on the conclusions of compositional analysis. They recommended using a minimum buffer size equal to the mean distance moved per day and stated that locational error should also be taken into account. Milksnakes moved an average of once every 5 days and they were tracked every 2 - 3 days. Therefore, for the majority of locations the snakes would not move between tracking days and I felt it was unnecessary to take into account the mean distance moved per day when calculating buffer size. I could locate the radio-implanted milksnakes within 1 m and, thus, there was virtually no radio-telemetry error. There was, however, an average of 5 m error in the GPS readings and a 5 m error in the satellite imagery. To correct for this, I made the radius of the buffer to equal 10 m. Habitat use was then calculated by the percentage of each habitat type within the 10 m buffer around each location. Habitat availability was calculated using the 95 % kernels.

Statistical Analyses

All statistical analyses were performed with R (R Development Core Team 2004) and JMP version 5.0.1 (SAS Institute 2002). I reported all means $\pm$ one standard error and accepted significance of tests at $\alpha = 0.05$.

Results

Macrohabitat Selection

I collected habitat data on 15 males and 8 females. I only used individuals, however, for which home range size reached an asymptote to ensure that the number of locations was sufficient to estimate total home range size. Based on this criterion, I used 9 males and 6 females in my macrohabitat analysis. Mean 95% kernel home range size (set to equal MCP size for each individual) for males (13.19 ± 2.68 ha) and females ($7.80 \text{ ha} \pm 3.28$ ha) were not significantly different ($t_{13} = 1.27$, $p = 0.23$).

For each kernel density I used a MANOVA to determine if habitat selection differed between the sexes. For the 95% ($F_{1,39} < 0.08$, $p = 0.07$), 75% ($F_{1,39} = 0.05$, $p = 0.14$), and 50% ($F_{1,39} = 0.016$, $p = 0.42$) kernels there was no difference between males and females, but habitat selection was non random for all three kernel density estimators (95% - $F_{1,39} = 0.17$, $p = 0.011$, 75% - $F_{1,39} = 0.14$, $p = 0.02$, 50% - $F_{1,39} = 0.17$, $p = 0.012$).

In all three kernels, milksnakes selected rocky outcrops and fields more than available and marshes and forests less than available. This trend increased as the density of the kernel estimator decreased (Fig. 2-1). Thus, milksnakes select rocky outcrops and fields at the macrohabitat level, and this is particularly true in core areas. When comparing between pairs of habitat types in the 95% kernel, the rankings were; rocky outcrop > field > marsh > forest. There was no significant difference between the top two or bottom two habitats (Table 2-4). In the 75 % and 50% kernels, the rankings were similar; rocky outcrops > fields > forests > marshes. In these two kernels, however, there were no significant differences between pairs, despite the differences between use and availability being greater (Table 2-4). My sample size is small and, therefore, the increased variability with lower kernel density (smaller size) decreased the power of the tests.

There was no difference in length of edge per hectare between males and females for the 95% ($t_{16} = 1.10$, $D = 23.12$, $p = 0.29$), 75% ($t_{16} = 0.135$, $D = 5.74$, $p = 0.89$), or the 50% ($t_{16} = 0.89$, $D = 8.15$, $p = 0.90$) kernel density. Therefore, I pooled the data for males and females and tested if milksnakes had a preference for edges in each kernel density. In the 95% ($t_{17} = 4.64$, $D = 47.94$, $p < 0.0001$), and in the 75% ($t_{17} = 3.30$, $D = 66.15$, $p = 0.0043$) kernels, milksnakes had more edge habitat in their home range than available. In the 50% kernel, they also had more edge habitat, but this difference was not significant ($t_{17} = 1.63$, D

= 45.97, $p = 0.12$). The magnitude of the difference between edge included in the home range and available edge was similar to the 95 and 75 % kernel, but again there was more variability in the smaller area, decreasing the power of the tests.

Microhabitat Selection Based on Land Cover

To be able to make direct comparisons between selection at the macrohabitat level and the microhabitat level, I used the same snakes and locations in both analyses. Based on the general habitat types recorded during the field surveys, male and female milksnakes selected habitats differently ($F_{1,39} = 0.15$, $p = 0.02$). Therefore, I analyzed males and females separately. Both females ($F_{1,15} = 2.10$, $p < 0.001$) and males ($F_{1,24} = 8.62$, $p < 0.001$) selected habitat non-randomly within the home range. Males and females used fields and rocky outcrops more than available, and forests and marshes less than available (Fig. 2-2). Using log ratios, the order of selection for males was: rocky outcrop > field > forest > marsh. The order of selection for females was: field > rocky outcrop > forest > marsh (Table 2-5). Males preferred rocky outcrops most, while females preferred fields most. There was no significant difference, however, between the two highest ranking habitats for both males and females. Also, for males forests were selected significantly more than marshes, while for females this difference was not significant, despite the difference between use and availability being similar (Fig. 2-2).

Microhabitat Selection Based on Habitat Characterization

I characterized the habitat at a total of 279 snake locations (166 male and 113 female) and their paired random locations from 23 individuals (15 males and 8 females). For both males and females, I selected the model with the lowest AIC value. The best model for males had two variables (%Rock and Dedge) that were not included in the best model for females

and, similarly, Nlog was in the best model for females but not in the best model for males (Table 2-6). To be able to make comparisons between the sexes, I included the missing variables in each model. The inclusion of the additional variables increased the AIC by 1 for males (128 – 129) and three for females (91-94). There were also only small changes in the original coefficients relative to their variability (Table 2-6). Therefore, I left the additional variables in the models to make comparisons between the sexes.

Both the model for male ($D_{7,166} = 115$, $R^2 = 0.29$, $p < 0.0001$) and the model for female ($D_{7,166} = 76.7$, $R^2 = 0.29$, $p < 0.0001$) milksnakes were significant. Based on the odds ratios, male milksnakes were using locations with open canopy that were close to edges: a 10% increase in canopy closure resulted in a 19% decrease in the probability of selection and, similarly, a 10 m increase in distance from an edge resulted in a 24 % decrease in probability of selection (Table 2-7). Male milksnakes also demonstrated a strong preference for rocky habitats. A size increase of 10 cm of the nearest boulder resulted in a 22% increase in the probability of selection. Increasing the number of boulders within 5 m of the location by 5 led to an 11% increase in the probability of selection. A 10% increase in the % Rock at the snake location led to 38 % increase in the probability of selection. Finally, male milksnakes avoided leaf cover: a 10 % increase in %Leaf led to a 22% decrease in the probability of selection.

Female milksnakes exhibited similar selection patterns as males (Table 2-6). Females preferred open canopy: a 10% increase in canopy closure led to a 27% decrease in the probability of selection. Females selected locations with more boulders (adding 5 boulders led to a 30% increase in the probability of selection) and larger rocks (a 10 cm increase in the size of the closest rock led to 14% increase in the probability of selection). Like males,

female milksnakes avoided leaf cover: a 10% increase in leaf cover lead to a 24% decrease in the probability of selection. Females also disliked many logs: adding 5 logs led to a decrease in the probability of selection of 50%.

There were some differences in the selection patterns of males and females. None of the differences in odd ratios, however, were outside the 95 % confidence intervals and all the coefficients (except Nlog) had the same sign (Table 2-7). Nlog, however, was very variable for both males and females (Table 2-7).

Comparison of Remote Sensing and Traditional Approaches

For this analysis, I again used the same snakes and locations that I used in the macrohabitat analysis. Using 10 m buffers around locations, there was a significant difference in habitat selection between the sexes ($F_{1,39} = 0.16$, $p = 0.01$). Therefore, I analyzed the sexes separately. Females selected the habitat within their home range randomly ($F_{1,15} = 0.19$, $p = 0.19$), while males selected the habitat non-randomly ($F_{1,24} = 0.22$, $p = 0.03$). Males used rocky outcrops and fields more than available and forest and marshes less than available (Fig. 2-3). Comparing the log ratios between pairs of habitats, the rankings were: rocky outcrop > field > forest > marsh. These are the same rankings that I obtained for the males from the ground data. None of the differences between rankings were significant, however.

Discussion

Hierarchical Habitat Selection

In Chapter 1, I demonstrated that thermal quality was an important factor influencing milksnake behaviour and habitat use. Because of its importance in this northern population, I predicted that thermal quality would influence habitat selection at all scales: influencing

selection both at the home range scale and at the scale of the location. As predicted, milksnakes used the habitat non-randomly at all spatial scales examined and preferred habitats of high thermal quality. The most parsimonious explanation for the patterns observed is that milksnakes selected macrohabitat and microhabitat that facilitate thermoregulation.

At the macrohabitat scale, milksnakes preferred fields and rocky outcrops over forests and marshes. Within their home range, milksnakes also selected more locations in fields and rocky outcrops than were available, and were selecting them significantly more than forests and marshes. In chapter 1, I demonstrated that open habitats (fields, rocky outcrops, marshes) have higher thermal quality than forested habitats. Milksnakes, therefore, prefer habitats of high thermal quality at both scales. Interestingly, however, milksnakes did not prefer marshes at any scale and marshes also have high thermal quality. This discrepancy could be related to foraging. Milksnakes' main prey are small mammals (Williams 1988) and it is unlikely that such prey occur in marshes. In Chapter 1, I showed that mammalian prey of milksnakes were more abundant in forest than in fields. Despite the lower abundance of prey in fields, milksnakes preferred fields over forests at all scales, outlining the importance of thermal quality in habitat selection patterns of snakes.

Milksnakes prefer edges at both micro and macrohabitat scales. At the macrohabitat scale, they had more edge per hectare in their home range than was available and habitat characterizations revealed that they selected locations that were close to edges. In edges milksnakes have access to both open and forested habitats with very short movements, which would be beneficial for behavioural thermoregulation. Therefore, edges offer the high prey abundance typical of forest and the high thermal quality typical of fields and rocky outcrops.

With matched-pairs logistic regression at the microhabitat level, I confirmed that milksnakes preferred locations with open canopy and avoided forests. This again suggests that milksnakes select habitats that facilitate behavioural thermoregulation. Milksnakes also prefer locations with many boulders and close to large rocks. Milksnakes used boulders and rock piles frequently as retreat sites and were often located under or beside them. In Chapter 1, I showed that in direct sunlight the operative environmental temperature was often above the preferred body temperature of milksnakes. By selecting rocky locations in the open, milksnakes have access to the open habitat for basking, but can retreat easily when body temperatures get too high or for protection against predators.

Milksnakes selected the habitat non-randomly at both macro and microhabitat scales. This highlights the importance of studying habitat selection at multiple spatial scales to gain a more complete understanding of selection patterns. When studying habitat selection at multiple spatial scales, there are often differing (Orians and Wittenberger 1991, Luck 2002) and sometimes conflicting (Compton et al. 2002) patterns. This has been suggested to result from differing selection pressures and limiting factors at different spatial scales (Rettie and Messier 2000). For milksnakes, thermoregulation seemed to be the most important factor driving habitat selection at both spatial scales studied. Other factors such as foraging and predator avoidance, however, cannot be ruled out.

Remote Sensing

Analysis of habitat selection with remote sensing provides an alternative to field surveys that are both time consuming and expensive (Fuller et al. 1989). The major challenge with remote sensing is that the scale of interest is often smaller than what can be resolved with the remotely sensed imagery. As the resolution of available satellite imagery increases,

researchers attempt to apply it to finer scales. Using 20-25 m resolution satellite imagery, habitat use analyses were sufficient to obtain coarse estimates of habitat selection and species-area relationships (Mack et al. 1997, Saveraid et al. 2001). This approach, however, could not detect habitat patches smaller than 1 ha and, thus, was not as accurate as field surveys.

Here, I tested the ability of IKONOS imagery with a resolution of 4 m to characterize habitat selection at the location scale in milksnakes. Based on field surveys, I demonstrated non-random habitat selection by males and females. When the same locations were classified with 10 m buffers using the imagery, the general patterns of selection were the same, but the significance levels were different. Only males were shown to select the habitat non-randomly and, although the rankings were the same, none of the habitat pairs were significantly different.

These discrepancies demonstrate that the results obtained with the remotely sensed images are not as reliable as field surveys to investigate habitat selection at the scale of the location. The differences were due largely to small changes in percentages of habitat use and to a slightly higher variability. The sample sizes were relatively small in this study and, thus, the power was low in many analyses. Because the trends were the same in both approaches, however, it would be interesting to test whether the patterns would become identical with a larger sample sizes.

Based on these preliminary results, using high resolution satellite imagery is promising for habitat selection studies at the scale of individual locations. Because of the small differences observed, however, it is important to compare the results with field surveys to determine if the trends are the same and the sample size required to obtain valid results.

Conclusion

Habitat selection studies that only examine selection at one scale are limited because different factors can influence selection at different scales (Rettie and Messier 2000). Many studies have demonstrated the importance of studying habitat selection at multiple scales (Orians and Wittenberger 1991, Compton et al. 2002, Luck 2002). In very few studies of reptiles, however, has second order selection been investigated. This is surprising because reptiles, and snakes in particular, are ideal organisms to study hierarchical habitat selection. First, unlike mammals that are easily disturbed, reptiles can be located within 1 m without disruption. Therefore, they do not have to be located using triangulation, which lead to large error in position. Second, snakes do not move far and often use the same location more than once. Therefore, locations every 2 - 3 days throughout the study season are sufficient to ensure that the entire home range is estimated.

In this study, snakes preferred habitats with the highest thermal quality. The most parsimonious explanation for this pattern is that snakes use these habitats to facilitate behavioural thermoregulation. Thermal quality was most likely so strongly linked to habitat use because I 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 explain better observed selection patterns. Similarly, the effects of reproductive state, season, and level of disturbance on habitat selection could be examined at multiple scales with sufficient sample sizes.

Table 2-1. Accuracy of classification for each habitat type based on locations selected on the imagery that were verified on the ground. Classification was performed on IKONOS imagery with 4 m resolution.

Habitat	Number of Points	Percent Accuracy
Field	20	90
Forest	19	95
Rocky Outcrop	19	89
Marsh	22	77
Open Water	18	100

Table 2-2. Outline of the matrix used to compare habitat use between habitat types and establish habitat rankings. The matrix establishes rank by comparing use to availability between pairs of habitat types. A positive value represents greater usage of the numerator relative to the denominator. Therefore, the number of positive values is used to determine rank. Modified form Aebischer et al. (1993).

Habitat types (numerator)	Habitat types (denominator)				Positive values (total)
	¹ Field	² Rocky Outcrop	³ Marsh	⁴ Forest	
¹ Field		$\ln(1_u/2_u) - \ln(1_A/2_A)$	$\ln(1_u/3_u) - \ln(1_A/3_A)$	$\ln(1_u/4_u) - \ln(1_A/4_A)$	
² Rocky Outcrop	$\ln(2_u/1_u) - \ln(2_A/1_A)$		$\ln(2_u/3_u) - \ln(2_A/3_A)$	$\ln(2_u/4_u) - \ln(2_A/4_A)$	
³ Marsh	$\ln(3_u/1_u) - \ln(3_A/1_A)$	$\ln(2_u/3_u) - \ln(2_A/3_A)$		$\ln(3_u/4_u) - \ln(3_A/4_A)$	
⁴ Forest	$\ln(4_u/1_u) - \ln(4_A/1_A)$	$\ln(2_u/4_u) - \ln(2_A/4_A)$	$\ln(3_u/4_u) - \ln(3_A/4_A)$		

U = use

A = availability

Table 2-3. Structural variables used in the analysis of habitat selection by eastern milksnakes in Ontario (2003 and 2004) with associated abbreviations and sampling radii.

Variable	Radius (m)	Description
Drock	20	Distance (m) to nearest rock (> 20 cm length)
Lboulder	20	Length of nearest rock (m) (> 20 cm length)
Dlog	20	Distance (m) to nearest log (> 7.5 cm diameter)
Dialog	20	Diameter (m) of nearest log (> 7.5 cm diameter)
Llog	20	Length (m) of nearest log
Dunder	20	Distance (m) to nearest understory tree (> 2m 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 Dbh and > 2m
7.5-15	10	Number of trees > 7.5 and < 15 cm Dbh in plot
15-30	10	Number of trees > 15 cm and < 30 cm Dbh in plot
30-45	10	Number of trees > 30 cm and < 45 cm Dbh in plot
>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
%Log	1	Coverage (%) of logs within plot

Table 2-3: Continued

%Grass	1	Coverage (%) of grass within plot
%Shrubs	1	Coverage (%) of shrubs within plot
%Herbs	1	Coverage (%) of herbs (non-woody) within plot
%Soil	1	Coverage (%) bare soil within plot
%Sticks	1	Coverage (%) sticks within plot
CanClo	45°	Canopy closure (%) within cone

Dbh = diameter at breast height.

Table 2-4. Matrices of t-values and associated p-values comparing use between pairs of habitat types for 3 kernel home range estimators generated from eastern milksnake locations in Ontario (2003 and 2004). Preference rankings are in order of most (1) to least preferred (4).

Habitat	Field	Rocky	Marsh	Forest	Rank
	Outcrop				
95% Kernel					
Field		-0.25	2.7	3.8	2
		0.80	0.020	0.0020	
Rocky Outcrop			2.4	3.6	1
			0.030	0.0030	
Marsh				0.71	3
				0.45	
Forest					4
75% Kernel					
Field		-0.24	2.4	1.7	2
		0.81	0.030	0.11	
Rocky Outcrop			1.69	2.1	1
			0.11	0.050	
Marsh				-0.59	4
				0.56	
Forest					3

Table 2-4. Continued

50% Kernel				
Field	-0.12	1.5	0.71	2
	0.90	0.15	0.49	
Rocky Outcrop		2.00	1.0	1
		0.06	0.31	
Marsh			-1.7	4
			0.12	
Forest				3

Table 2-5. Matrices of t-values and associated p-values comparing use between pairs of habitat types for eastern milksnake locations in Ontario (2003 and 2004). Preference rankings are in order of most (1) to least (4) preferred.

Habitat	Field	Rocky	Marsh	Forest	Rank
		Outcrop			
Female					
Field		0.86	4.8	2.7	1
		0.43	0.0040	0.040	
Rocky Outcrop			3.2	0.70	2
			0.02	0.51	
Marsh				-2.0	4
				0.10	
Forest					3
Male					
Field		-1.5	10	5.7	2
		0.17	<0.001	<0.001	
RO			8.7	7.6	1
			<0.001	<0.001	
Marsh				-6.3	4
				<0.001	
Forest					3

Table 2-6. Paired logistic regression models of microhabitat use by eastern milksnakes in Ontario (2003 and 2004).

Male		Coefficients					
AIC	Lboulder	Nboulder	CanClo	Dedge	%Rock	%Leaf	Nlog
128	2.1	0.021	-0.021	-0.027	0.032	-0.024	
129	2.1	0.021	-0.021	-0.028	0.032	-0.025	0.04
Female							
91	1.5	0.062	-0.031			-0.028	-0.15
94	1.3	0.056	-0.031	-0.004	0.13	-0.027	-0.15

Table 2-7. Paired logistic regression models for male and female milksnakes in Ontario (2003 and 2004).

Male					
Variable	Coefficient	Std. Err.	Increase	Odds Ratio	95% CI (OR)
Lboulder	2.1	0.92	10 cm	1.2	(1.0, 1.4)
Nboulder	0.021	0.011	5 boulders	1.1	(1.0, 1.2)
CanClo	-0.021	0.007	10%	0.81	(0.67, 0.95)
Dedge	-0.028	0.011	10m	0.76	(0.55, 0.98)
Rock	0.032	0.015	10%	1.38	(1.1, 1.7)
Leaf	-0.025	0.012	10%	0.78	(0.56, 1.0)
Nlog	0.040	0.070	5 logs	1.22	(0.53, 1.9)
Female					
Lboulder	1.3	0.94	10cm	1.14	(0.95, 1.3)
Nboulder	0.052	0.020	5 boulders	1.30	(1.1, 1.5)
CanClo	-0.031	0.0090	10%	0.73	(0.55, 0.91)
Dedge	-0.0040	0.011	10m	0.96	(0.74, 1.2)
Rock	0.013	0.016	10%	1.14	(0.82, 1.4)
Leaf	-0.027	0.015	10%	0.76	(0.47, 1.1)
Nlog	-0.15	0.085	5 logs	0.50	(-0.35, 1.3)

Table 2-8. Matrices of t-values and associated p-values comparing use between pairs of habitat types for eastern milksnake locations in Ontario (2003 and 2004). Location data was collected using 10 m buffers collected by remote sensing. Preference rankings are in order of most (1) to least (4) preferred.

Habitat	Field	Rocky	Marsh	Forest	Rank
	Outcrop				
Field		-0.67	1.5	0.29	2
		0.51	0.18	0.77	
Rocky Outcrop			1.9	2.2	1
			0.090	0.06	
Marsh				-0.67	4
				0.50	
Forest					3

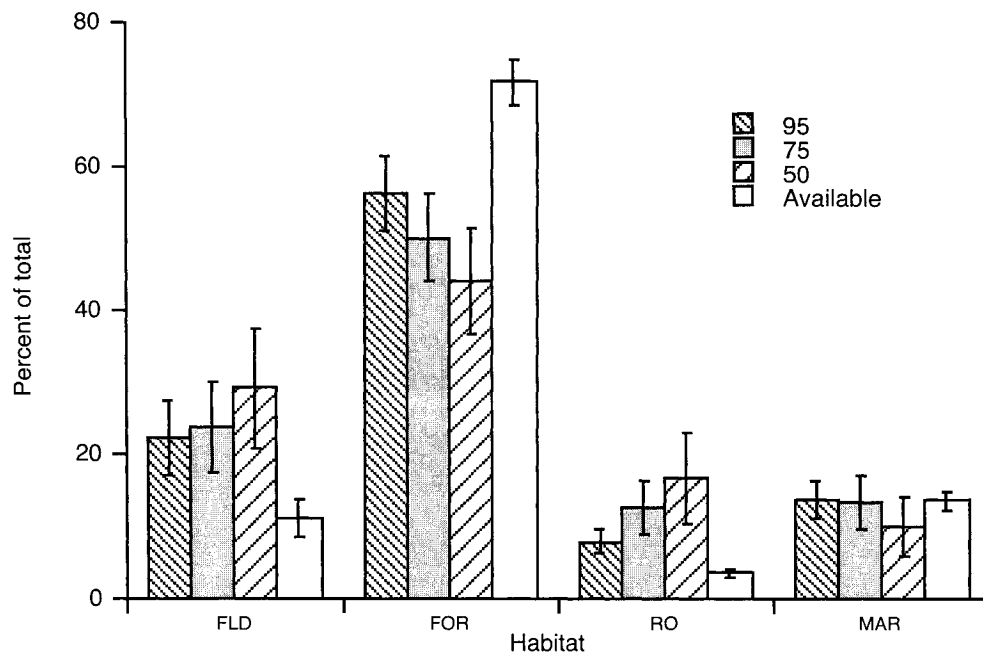


Fig. 2-1. Percentage of four habitat types within varying kernel home range estimators and available habitat for eastern milksnakes in Ontario (2003 and 2004).

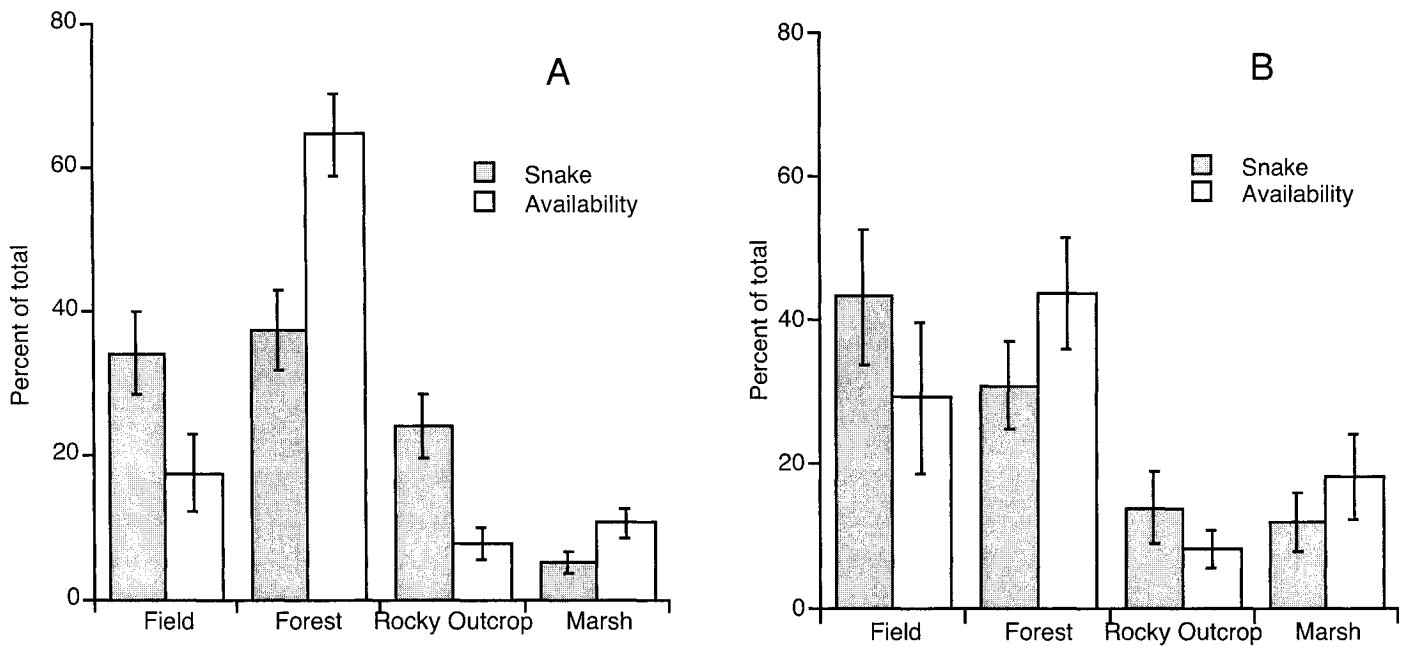


Fig. 2-2. Mean percentage of locations ($\pm$ SE) in each of four habitat types for A) male and B) female milksnakes in Ontario (2003 and 2004) based on field surveys. Available habitat is the mean percentage of each habitat type ($\pm$ SE) within the 95% kernel home range estimators.

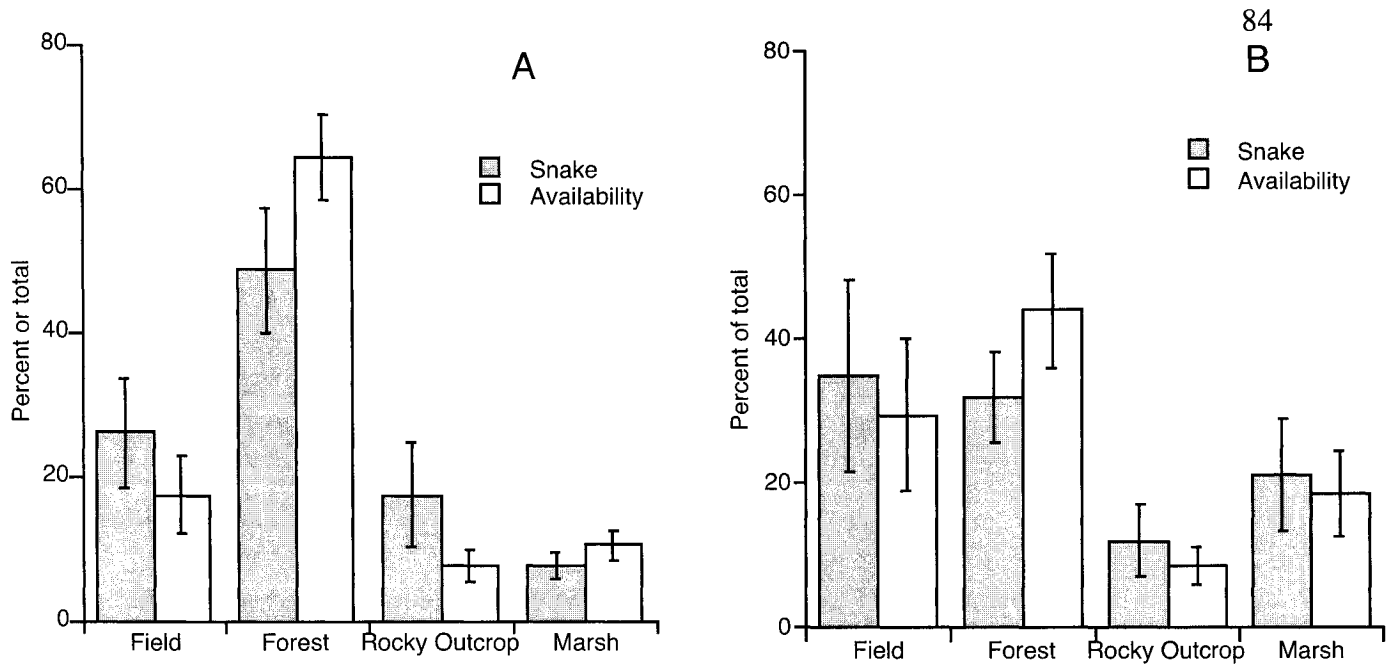


Fig. 2-3. Mean percentage of 10 m buffers ($\pm$ SE) in each of four habitat types for A) male and B) female milksnakes in Ontario (2003 and 2004) based on satellite imagery. Available habitat is the mean percentage of each habitat type ($\pm$ SE) within the 95% kernel home range estimators.

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