Thermoregulation and habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) at the northern extreme of their distribution

by

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ABSTRACT

The central goal of this study was to understand how black rat snakes met the thermal challenges they faced living at the northern extreme of their distribution. In Chapter One, I demonstrated that all black rat snakes prefer edge habitats, but that the preference for edges was strongest for gravid females. I hypothesised that edges were preferred because of the superior thermoregulatory opportunities they provide. Because females did not feed while gravid, their use of edges appeared to be unrelated to foraging, and because retreat sites were as numerous in forest as in edges, the snakes were unlikely to have been using edges primarily to avoid predators.

In Chapter Two, I showed that gravid female black rat snakes thermoregulated more effectively and exploited their thermal environment more than non-gravid females and males. Also, despite their challenging thermal environment, black rat snakes did not thermoregulate less than species living under more benign climates. This result was contrary to what would be expected based on the cost-benefit model of thermoregulation.

In Chapter Three, I showed that edges had the highest thermal quality of all the habitats available to black rat snakes. However, black rat snakes experienced body temperatures closer to their preferred body temperature range while in barns than while in edges and forest, and invested less in thermoregulation while in edges than while in barns and forest. Thus,

contrary to the cost-benefit model of thermoregulation, the extent of thermoregulation in a given habitat is not a simple function of the habitat's thermal properties.

In Chapter Four, I demonstrated that black rat snakes increased thermoregulation after feeding, both in the laboratory and in the wild. After being fed in the wild, black rat snakes were more likely to be found in edges. These results provide experimental support for the hypothesis that edges are used for thermoregulation.

In Appendix One, I described the anaesthesia and surgical procedures I used to implant radio-transmitters in black rat snakes. This information will benefit anyone initiating radio-telemetry on black rat snakes or other closely related species.

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"Toute la mer monte

pour une seule pierre qu'on y jette."

Blaise Pascal [1623-1662]

Liberal translation :

"The whole sea rises

for a single stone that we throw in."

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CHAPTER ONE

Habitat selection in black rat snakes (*Elaphe obsoleta obsoleta*)

This chapter formed the basis for the following publication:

Blouin-Demers, G. and P. J. Weatherhead. In press. Habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. Ecology.

Introduction

Habitat loss and habitat fragmentation are the two main threats to biodiversity (Wilson 1992). Habitat loss has direct consequences for species abundance and diversity because it reduces the available living space. The consequences of fragmentation are more complex and depend upon the degree of fragmentation, the shape and spatial arrangement of the fragments, and the composition of the separating habitat. The primary impact of fragmentation is the isolation of populations. Isolation enhances the chances of stochastic events causing local extinction and also reduces gene flow. The secondary impact of fragmentation is to increase the proportion of edge habitats in the landscape. Increasing edge habitats can further affect ecosystems because it modifies ecological relationships such as predator-prey interactions (Donovan et al. 1995).

The majority of research on forest fragmentation has focused on the consequences for species that are adversely affected and has often used birds as the model system (McCollin 1998). Fragmentation is thought to be one of the key factors responsible for the decline of many bird species (Bolger et al. 1991, Donovan et al. 1995). There are two main explanations for these declines. First, as forest fragments get smaller, at some point they no longer have true forest interior habitat, and thus are no longer suitable for forest-interior species. Second, nest success declines in forest fragments because the density

or success of generalist and edge-associated predators increases (Yahner and Scott 1988, Andrén 1992) or brood-parasitism increases (Brittingham and Temple 1983, Robinson et al. 1995). Nest predation is the most significant cause of nest failure in birds (Ricklefs 1969, Martin 1988a,b) and has been demonstrated to increase dramatically with proximity to edges (Andrén and Angelstam 1988, Burkey 1993). Although birds are often identified as nest predators (Angelstam 1986, Yahner and Cypher 1987), mammals (Hoffmann and Gottschang 1977, Small and Hunter 1988) and snakes (Loiselle and Hoppes 1983, Robinson et al. 1995) are also important.

More detailed information about the use of habitat edges by predators is a prerequisite for a better understanding of the interrelation between landscape configuration and the importance of predation in local animal communities (Angelstam 1986). Because black rat snakes are important nest predators (Fitch 1963, Stickel et al. 1980), if the increase in edge habitats caused by fragmentation increases rat snake abundance or hunting success, then black rat snakes can play an important role in the predation dynamics of fragmented ecosystems. My central objective in this chapter was to investigate habitat selection patterns of black rat snakes, and in particular their response to habitat edges, as a first step in understanding the underlying causes of habitat selection in this predator.

All ectothermic animals obtain heat from their environment, resulting in their population biology being characterised by low energy flow and

efficient biomass production (Pough 1980). Body temperature regulation is achieved by adjusting microhabitat selection and timing of activity (Patterson and Davies 1982, Huey et al. 1989, Krohmer 1989). Thermoregulation is probably the single most important proximate factor in the habitat selection process and timing of activity of terrestrial squamates (Grant 1990, Reinert 1993), with the probable exception of those living in tropical climates (Shine and Madsen 1996).

Gravid females of many viviparous snake species have been shown to thermoregulate differently from non-gravid females and males (Charland and Gregory 1990, Cobb and Peterson 1991, Brown and Weatherhead 2000). This difference in thermal preference was also shown to be associated with altered habitat preferences, with gravid females favouring habitats providing better opportunities for thermoregulation (Reinert 1984b, Reinert and Zappalorti 1988). Although the same type of behaviour has been suggested to occur in oviparous species (Shine and Madsen 1996), it has not been formally documented. My first goal in this chapter was to contrast the habitat selection patterns of male, non-gravid female, and gravid female black rat snakes. I predicted that reproductive females of this oviparous species would exhibit patterns of habitat use similar to gravid females of viviparous species: a greater preference for habitats providing the best opportunities for thermoregulation compared with males and non-gravid females. Alternative explanations for the different behaviour of gravid females could be that the

impaired mobility of females when gravid (Seigel et al. 1987) forces them to alter their choice of prey, or to rely more heavily on refuges from predators, both of which could result in altered habitat selection. If gravid females use habitat different from that used by other snake groups because they change diet, then I predicted that their post-egg-laying weight should not show evidence of fasting. Also, they should exhibit some evidence of foraging by changing location from day to day. If gravid females use different habitats to avoid predators (Cooper et al. 1990, Charland and Gregory 1995), then I predicted that gravid females should be concealed more often than the other snake groups and should exhibit evidence of fasting.

Only two studies of habitat use in black rat snakes have been published (Weatherhead and Charland 1985, Durner and Gates 1993). Both studies showed that black rat snakes prefer edge habitats. However, both studies were limited in two ways. First, both were conducted in landscapes where the total availability of edge habitat had been increased substantially by human activity (e.g., forest clearing for agriculture). Therefore, the detected preference for edge habitats could have been an artefact of the fragmented study areas. Second, both studies involved relatively small numbers of snakes (7 and 31, respectively) followed for relatively short time periods (3 and 5 months, respectively). In this study I collected data from 52 individuals tracked for periods of up to 41 months. To identify the factors that influence habitat use in this species, I needed to evaluate the types of habitats used by black rat

snakes under natural conditions to determine if an association with edges also occurs in more natural habitats. The use of edges in human-disturbed landscapes by black rat snakes may reflect a pre-existing preference for edges in natural landscapes. Alternatively, the human-induced habitat changes may have altered rat snake habitat preferences and created habitats that were invaded by this species. My second goal was to test the prediction that black rat snakes in pristine landscapes also prefer edges, where the edges are natural boundaries between habitats, such as marsh borders, or rock outcrops in otherwise continuous forest.

To identify the causes of habitat preference, it is useful to investigate seasonal variation in habitat use. If habitat preference is highly seasonal in black rat snakes, it might provide insights into the factors that determine habitat selection. For example, Weatherhead and Charland (1985) found that the preference for edges in their study was most pronounced during the spring. They hypothesised that black rat snakes used those habitats preferentially to take advantage of the high density of breeding birds (a prey item for rat snakes) in ecotones during the spring, although they could not rule out the possibility that they used edges for thermoregulation. My third goal was to investigate whether there was a seasonal preference for edge habitat by black rat snakes, and if such a preference was found, whether that preference was more consistent with the exploitation of seasonal prey

abundance in edges, or with seasonal variation in thermoregulatory demands.

My final goal was to relate my results to the management of black rat snakes. My study population of black rat snakes occurs as a separate population at the northern extreme of the species' range in eastern Ontario. Recently this population has been classified as threatened in Canada (Prior and Weatherhead 1998). Conservation efforts aimed at improving habitat for this species that rely on the two existing studies of habitat selection (Weatherhead and Charland 1985, Durner and Gates 1993) would presumably involve preserving or even creating edge habitats. Such an approach would conflict directly with conservation efforts for many threatened or endangered forest species that suffer from forest fragmentation. The first step toward resolving this potential conflict is to be certain that a conflict exists. By determining habitat preferences of black rat snakes under natural conditions and the factors that influence habitat use, this study will provide an empirical basis necessary for sound management.

Materials and methods

This research was conducted from 1996 to 1999 in the immediate vicinity of the Queen's University Biological Station on the shores of Lake Opinicon near Chaffey's Locks in eastern Ontario (44° 34'N, 76°, 19'W). The study area was approximately 9.5 km by 2.5 km and encompassed the

Biological Station. The dominant geological feature of the area is the exposed south eastern extension of the Canadian Shield referred to as the Frontenac Axis. This area is characterised by strongly rolling terrain with ridges of granite outcrops alternating with valleys every 500 m and numerous small lakes and wetlands. The study area is mostly second growth forest dominated by deciduous trees (*Acer, Quercus, Ostrya, Populus, Fraxinus, Carya, Ulmus, Fagus, Tillia, Betula*), although some coniferous trees were present in the drier, sandier soils (*Pinus, Thuja, Tsuga, Abies*). In addition, the ongoing abandonment of marginal farmland has resulted in old fields and scrub habitats (*Zanthoxylum, Juniperus*) being common. Some of the better fields are still hayed.

To obtain experimental animals, I captured snakes at 13 communal hibernacula. The hibernacula had been located by radio tracking snakes to their hibernation sites, both as part of previous studies of black rat snakes (Weatherhead and Charland 1985, Weatherhead and Hoysak 1989) and as part of the present study. I selected hibernacula as a source for the experimental animals for this study that were centrally located in the study area. This increased the likelihood that the snakes would remain in the study area during the active season, although I was prepared to modify the exact boundaries of the study area to accommodate the snakes as necessary.

To capture emerging rat snakes, I constructed perimeter fences by stapling polyethylene plastic to a 1.5 m high timber frame of posts and rails

built around hibernacula openings. I buried the bottom edge of the plastic and installed a funnel trap in one corner of the fence. These fences were supplemented by careful searches of the ground and trees around all hibernacula because the fences were not totally escape-proof and, in some instances, all the openings at a given hibernaculum could not be enclosed because their location was unknown or the terrain prevented it. Traps were installed in early April and site visits begun prior to the start of emergence each spring (Blouin-Demers et al. 2000b) and continued until the last snake emerged (late May).

Snakes captured at all sites were processed in a similar manner. Upon capture, snakes were sexed by gently probing for the presence of hemipenes, measured for snout-vent length (SVL) to the nearest 1 mm with a metric tape, weighed to the nearest 1 g with a calibrated spring-scale, and marked by a subcutaneous injection of a passive integrated transponder (PIT) tag (Anitech Identification Systems Inc., Markham, Ontario).

I selected study animals from among all the animals I captured to have radio-transmitters surgically implanted. My choice of individuals was based on sex and size. Snakes had to be large enough to bear the transmitter and, because females reproduce every second to third year on average (G. Blouin-Demers, unpublished data), I had to implant more females than males to obtain an adequate sample of gravid females. I used isoflurane delivered via a precision vaporiser to anaesthetise the snakes and then sterile surgical

techniques to implant the radio-transmitters in the body cavity and the antennae just underneath the epidermis. The surgical technique was modified from the procedure presented by Reinert and Cundall (1982). However, the anaesthesia procedures had to be developed for this study because very little information regarding reptilian anaesthesia is available in the literature. Also, preliminary trials indicated that black rat snakes did not respond well to the few procedures that were published. In Appendix One I present the detailed anaesthesia and surgical procedures I used, as well as an analysis of the effect of size and sex on induction and recovery times in black rat snakes.

After release, I located the snakes on average every 48 hrs using a telemetry receiver (TRX-2000S, Wildlife Materials Inc., Carbondale, Illinois) and a directional three-element antenna (F173-3FB, Wildlife Materials Inc., Carbondale, Illinois), from their emergence in late April until they re-entered their hibernacula in early October. Upon locating a snake, I recorded its location, position, and behaviour (concealed, resting/basking, or travelling). All snake locations were flagged and later mapped using the software MC-V Asset Surveyor Version 3.16 in a GPS Pathfinder unit equipped with a Probeacon for instantaneous differential correction (Trimble Navigation Ltd, Sunnyvale, California) that gives sub-meter accuracy in the field. The UTM (NAD83 datum) co-ordinates of each snake location were used to calculate the distance moved between relocations. From May 1996 to October 1999, I

followed 23 males and 41 females for periods ranging from 16 days to 41 months, with 52 of those snakes (18 males and 34 females) followed for more than 3 months. Only the 52 individuals tracked for at least 3 months were used in the analysis of habitat selection.

Habitat characterisation

Locations where snakes were found actively travelling were excluded from the analysis of habitat selection to avoid including instances where snakes may have been disturbed by my approach and to make this study comparable to other studies of snake habitat selection (Reinert 1984a,b, 1992, Reinert 1993). This accounted for only 285 of 3847 (ca. 7%) locations. I also excluded instances where snakes were found in buildings because these sites could not be characterised adequately with my habitat sampling scheme (see below). Snakes were in buildings in 270 of 3847 (ca. 7%) relocations. For each snake, I only quantified the habitat at every second relocation to keep the habitat sampling manageable. I quantified the habitat at a given position only after the individual had moved to another location to minimise disturbance. Locations at which a snake was observed more than once were only included once in the analysis of habitat selection. To quantify the available habitat, which is necessary to determine whether the snakes were using habitat non-randomly, I repeated the same habitat analysis and characterisation at sites selected at random. I selected these sites by walking a randomly determined distance (10 to 200 paces, determined by a die with 20 faces and multiplying by

10) in a randomly selected direction (1 to 360°, determined by spinning the bearing dial disc on a compass) from snake locations. To keep the sample size manageable I only selected random habitat points from every fourth snake location.

To characterise the habitat at snake and random locations, I measured a set of 28 structural variables within circular plots of different radii, depending on the variable of interest, all centred at the snake or random location (Table 1-1, Table 1-2). I measured all the distance variables to the nearest 1 cm by having an observer stand in the centre of the plot holding one end of a 50 m measuring tape while I walked to the feature of interest. To evaluate percent ground cover and canopy closure, I used a sighting tube (a 50 cm X 2.5 cm piece of piping) with a cross-wire at one end. This was a modified version of Winkworth and Goodall's (1962) apparatus. I aimed the tube randomly 50 times in a 2 m radius plot and recorded the type of ground cover "hit" in the cross-wire. I then multiplied the number of "hits" for each cover type by 2 and recorded this as the percent ground cover. The same procedure was used 20 times at an angle >45° from horizontal to determine the percent canopy closure. I defined an edge as the boundary between an "open" or two-dimensional habitat (e.g., hay fields, rock outcrops, marshes) and a "closed" or three-dimensional habitat (e.g., deciduous forest, coniferous forest). These are the types of edges that are associated with a higher density of breeding birds (Gates and Gysel 1978), and that also provide a forest snake with

thermoregulatory opportunities. An additional benefit of this definition was that all edges (or habitat boundaries) were clear and it made measuring distances from edges an objective process.

After the 1996 field season, I ran a preliminary Multivariate Analysis of Variance (MANOVA) and associated Discriminant Function Analysis (DFA) to identify which variables were contributing to the multivariate group differences between random sites, male sites, non-gravid female sites, and gravid female sites (see Statistical analyses below). From 1997 to 1999, I discontinued sampling the 13 discriminant variables that had pooled within-group correlations with the discriminant functions ≤ 0.10 (Table 1-1). This was a conservative approach because variables that contributed significantly to group differences had correlations ≥ 0.25. I used a conservative approach to ensure that all potentially meaningful variables were retained for further sampling. From 1997 to 1999, I only measured the 15 structural variables retained after the preliminary analysis (Table 1-2).

At each snake or random location, I also recorded the general habitat type as either forest, field, marsh, rock outcrop, "natural edge" or "artificial" edge. I defined an "artificial" edge as having been created by humans (e.g., edge between field and forest), whereas "natural" edges were not the result of human intervention (e.g., edge between marsh and forest). I considered a snake to be in an edge when it was within 15 m of the boundary between the "open" habitat and the "closed" habitat.

Statistical analyses

I divided the snakes into three groups based on their reproductive status: males, non-gravid females, and gravid females. The reproductive state of females was assessed in June by palpating the oviducts for the presence of eggs and confirmed in July by nesting activity. Because female black rat snakes do not reproduce every year, some individuals changed groups from one year to the next. Because female black rat snakes very seldom move while gravid (see Results), fewer locations were obtained for this group. I used MANOVA to determine if there was a significant difference in habitat centroids of each group and DFA to examine along which axes the groups differed and which variables contributed the most to group separation.

An assumption common to all Analysis of Variance (ANOVA) models is that observations are sampled at random. While truly random samples of organisms are extremely hard to obtain in nature, this assumption is particularly dubious when radio-telemetry is used because many observations are derived from relatively few individuals. In such a case, an "aberrant" individual sampled repeatedly can severely bias the conclusions one would reach when treating each observation as an independent sample. In the present study, the individual snake sampled the most only accounted for 7.2% of the total snake sample, which indicates that no individual had the opportunity to unduly bias the group means. Another potential solution to this problem would have been to use mean habitat vectors for each

individual as the basis for analyses. However, using this approach usually does not change the conclusions of the analyses (Reinert 1984a,b) while not making use of all the information available, such as the variation found within individuals.

One assumption specific to MANOVA is the homogeneity of covariance matrices, usually tested using Box's test. This assumption is rarely met with habitat selection data because it would require that each segment of the population responds similarly to the different habitat variables (Reinert 1984a,b). If this assumption is violated and the sample sizes for each group differ substantially, biased tests of significance can result (Stevens 1996). Because female black rat snakes are not gravid each year and because they are very stationary while gravid, the number of locations for this group was approximately half that of the other groups. To ensure that this difference in sample sizes did not unduly bias my tests of significance, I also conducted my analyses using a computer-generated randomly-selected subset of my data designed to achieve equal sample sizes in each group. Because the results for all the analyses remained qualitatively unchanged (all the significant relationships remained significant, no new significant relationship appeared, and the variables contributing the most to group differences were the same), I only present the results of the analyses using the complete data set.

The analyses were conducted on JMP Version 3.2 (SAS Institute 1997) and SPSS Version 6.1 (SPSS 1995) on a Macintosh desktop computer. I

inspected Box plots to determine if the assumptions of normality and homogeneity of variance were upheld. The continuous habitat structure variables were log transformed to improve their adherence to the normality assumption. Significance of statistical tests was accepted at $\alpha = 0.05$, but marginally non-significant results are reported when deemed important. All means are reported ± one standard error.

Results

From 1996 to 1999, I sampled habitat characteristics at 165 random locations, 195 male locations, 190 non-gravid female locations, and 81 gravid female locations. The general habitat types for snake and random locations clearly indicate a preference by all snake groups for edge habitats, and an avoidance of water bodies (Table 1-3). The mean scores on each variable for the random locations and the three snake groups locations are presented in Table 1-4. In general, the movement patterns and behaviour of rat snakes were stereotypical. All snakes emerged from hibernation in late April to early May, dispersed from the hibernaculum to their home range a few days later, and went through a first ecdysis in late May. Then, in early to mid-June, males engaged in mate searching, which was reflected in their long distances moved and their high frequency of travelling, whereas females engaged in shorter, less frequent movements. In mid-June, females that became gravid moved to a retreat site generally situated in edge habitat where they stayed 3-4

weeks until they needed to lay their eggs in mid-July. During the same time, females that were not gravid continued and males resumed their more frequent short movements. Typical gravid female retreat sites were hollows in standing or fallen dead trees at the edge between forest and fields, rock outcrops, or marshes.

Before I used MANOVA to determine if there were multivariate group differences in habitat use, I verified that the assumption of homogeneity of covariance matrices was met. As expected with biological data (Reinert 1984a,b), the Box's test was significant (Box's M = 798.36, $F_{(360,351883.2)} = 2.11$, $p < 0.001$), indicating that the covariance matrices were heterogeneous. However, many authors have defended the heuristic value of multivariate methods despite the common violation of this assumption with ecological data (Pimentel 1979, Stevens 1996).

The overall MANOVA indicated that the habitat characteristics of the three groups of snakes and the randomly sampled points were significantly different (Wilk's $\Lambda = 0.630$, $F_{(45,1821.9)} = 6.80$, $p < 0.001$). Distances between group centroids in the discriminant space showed that males, non-gravid females, and gravid females all used habitat non-randomly (i.e., each group differed significantly from the random habitat samples, Table 1-5). Among the three classes of snakes, males and non-gravid females did not differ significantly from one another, while gravid females were significantly different from both other groups (Table 1-5). In addition, gravid females used habitat that

was the least available in the study area (largest distance in the discriminant space from the random group), followed by males, and then by non-gravid females.

To determine which variables contributed the most to the multivariate differences I observed between the group centroids, I conducted a DFA. This analysis summarises the multivariate group differences in a set of discriminant functions accounting for the largest possible proportion of the total variance. The maximum number of discriminant functions is the minimum of the number of classes (groups) minus one, or the number of variables minus one. Because I had four groups, the procedure derived three discriminant functions. Only the first discriminant function ($\chi^2_{(45)} = 283.3$, $p < 0.001$) and the second discriminant function ($\chi^2_{(28)} = 59.0$, $p < 0.001$) accounted for a significant amount of the total variation, with the first discriminant function explaining 81.7% of the total variation (Table 1-6).

I examined the pooled within-groups correlations with the discriminant functions for each habitat variable to see which discriminant variables were the most strongly correlated with the discriminant functions explaining the multivariate group differences. For the first function, the most important variables referred to the distance to a tree (overstory or understory), the distance to an edge, and to the percent ground cover of logs and shrubs. This function therefore can be interpreted as a gradient from forest interior habitat towards forest edges habitats. For the second function,

the size and number of trees in a 10 m radius, the distance to rocks, and the percent ground cover of logs and rocks were important (Table 1-6), indicating a gradient from small trees and many rocks towards large trees and few rocks.

Fig. 1-1 is a pictorial interpretation of habitat selection for the different snake groups in relation to the available habitat in the study area. The first discriminant function provides clear separation between the random group and the three snake groups. The available habitat in the study area was mostly forested with low ground cover of logs and shrubs, whereas snakes tended to be found near edges with extensive ground cover of shrubs and logs. Gravid females had the strongest preference for sites close to edges and trees with high ground cover of logs and shrubs, as illustrated by their position furthest from the random sites along the first discriminant function. The second discriminant function provided further separation between gravid females and the other two snake groups (males and non-gravid females), illustrating that gravid females were associated with larger trees and less with rocks than the other two groups.

The habitat use of males and non-gravid females did not differ significantly, suggesting that the differences I observed in habitat use among the three groups are not a function of the sex of the individual *per se*, but rather, a consequence of the reproductive state of females. To examine more formally if the difference in habitat use I observed between gravid females and the other two snake groups was due to the reproductive condition of the

females, I contrasted the habitat use of snakes followed in multiple years. I had sufficient data (≥ 10 characterised locations in each year) to test for multivariate differences in habitat use among years for three males and one female. The three males were followed in 1996 and in 1997 and, in each case, there was no significant differences between their habitat use in each year (Wilk's $\Lambda = 0.011$, $F_{(2,15)} = 11.74$, $p = 0.08$; Wilk's $\Lambda = 0.278$, $F_{(4,15)} = 0.69$, $p = 0.73$; Wilk's $\Lambda = 0.445$, $F_{(11,15)} = 0.91$, $p = 0.57$). The single female was followed in 1997 (non-gravid) and in 1998 (gravid) and there was a significant multivariate difference in her habitat use among years (Wilk's $\Lambda = 0.132$, $F_{(12,15)} = 5.25$, $p = 0.003$), with lower scores on the first axis (stronger preference for edges) when she was gravid than when she was non-gravid.

Gravid females could change their habitat use because they have higher thermoregulatory needs (Charland and Gregory 1990, Cobb and Peterson 1991). Alternatively, their habitat change could reflect a shift in diet or predator avoidance. If gravid females use edges because they exploit different prey, I predicted that their post-parturition weight should not show evidence of fasting. I compared the weight at emergence from hibernation in early May to the weight following parturition in July for 11 females. After egg-laying, all females demonstrated a very significant weight loss (mean = 108.8 g ± 12.7 or 21.3% ± 2.5 of postpartum mass) compared to their emergence weight (Paired $t_{(11)} = 8.56$, $p < 0.001$), which suggests that females did not feed when they were gravid. Furthermore, if females were foraging while gravid, I

expected them to move from day to day searching for prey. I divided the distances moved between relocations into four classes (0 m, 1 to 10 m, 11 to 100 m, and 101 to 1000m) and contrasted the distances moved between relocations for the three snake groups during the period prior to egg laying (1 June to 15 July). There was a significant difference in distance categories among groups ($\chi^2_{(3)} = 72.38, p < 0.001$). Gravid females moved significantly less between relocations than non-gravid females or males. Gravid females had not moved for 64% of the relocations and had moved 10 m or less in 75% of the relocations (Fig. 1-2). By contrast, non-gravid females had moved 10 m or less in 53% of the relocations and males in 47% of the relocations (Fig. 1-2).

Another possible explanation for the different habitat use of gravid females is that edges provide retreats from predators during the period when females are more vulnerable to predators because of the burden of their eggs. If females used edges to avoid predators, I expected them to be concealed more often than males or non-gravid females and to use sites that provide more cover. The DFA (above) did indicate that gravid females preferred sites with higher ground cover of shrubs and were found in trees more often than the other snake groups. To determine if gravid females were concealed more often than other snakes, I divided the behaviour of the snakes when located during the period prior to egg laying as either travelling, resting/basking, or concealed. There was a significant difference in the frequency of each behaviour among the three snake groups ($\chi^2_{(2)} = 23.46, p < 0.001$), with gravid

females tending to be concealed more often than males or non-gravid females (Fig. 1-3). Therefore, the habitat use and behaviour of gravid females supports the hypothesis that they try to avoid predators more actively than the other snake groups.

Having established that all snakes preferred edges, I then compared their use of "natural" edges and "artificial" edges. Snakes were found in edges approximately 80% of the time, whereas edges represented only approximately 20% of the available habitat, again demonstrating the strong preference for edge habitats (Table 1-7). Although "natural" edges were approximately 2.5 times more abundant than "artificial" edges in the study area, snakes were no more likely to be found in "artificial" edges than in "natural" edges, based on the availability of both types of edges in the study area ($\chi^2_{(1)} = 0.12$, $p = 0.73$, Table 1-7). Thus, the snakes appeared to be responding to the structural aspects of an edge, rather than anything relating to how the edge was created.

Finally, I examined if the preference for edges was constant over the duration of the active season. I divided the snake locations into edge ("natural" or "artificial") and non-edge habitat. My prediction was that if edges are used for foraging, their use should be more prevalent when prey availability is high in those habitats (during the spring bird breeding season). I determined the proportion of snake locations that were in edges for each month of the main active season (May to August). Earlier and later

relocations could not be included in this analysis because there were too few to analyse. Although snakes were found in edges more often than in all other habitat types combined throughout the active season, this analysis showed that their use of edges increased significantly over the active season ($\chi^2_{(3)}$ = 8.90, p = 0.03, Table 1-8).

Discussion

Weatherhead and Charland (1985) and Durner and Gates (1993) reported that black rat snakes preferred edge habitats in human-disturbed landscapes. This preference could have been an artefact of their fragmented study areas. However, in this chapter I have shown that the preference by black rat snakes for ecotones is also pronounced in more pristine landscapes where the edges occur naturally. This result confirms the importance of edge habitat to black rat snakes and illustrates that this preference also occurs in more natural environments.

When I investigated population segment differences in habitat preferences, I found that males and non-gravid females were not significantly different from one another, but were different from gravid females. Because the males and non-gravid females were not significantly different, it suggests that the differences I observed in habitat use among the three groups are not a function of the sex of the individual *per se*, but rather, a consequence of the reproductive state of females. The comparison of habitat use by the same

individuals in different years, although based on a small sample size, supported this view. Males did not differ significantly in habitat use between years, whereas the single female did differ significantly when she was gravid and when she was not. Gravid females of viviparous species have been shown to prefer habitats offering the best opportunities for behavioural thermoregulation (Reinert 1984b). Effective thermoregulation is important for gravid females because the phenotype and fitness of their offspring are highly dependent upon the body temperatures they experience during development (Alberts et al. 1997, Shine et al. 1997a, b, Blouin-Demers et al. 2000a). However, this type of behaviour has not been formally demonstrated in oviparous species. In this chapter, I have shown that in an oviparous species, gravid females selected habitats that differed from the habitats used by non-gravid females and males, and that was most different among the three groups of snakes from the random habitat samples. Gravid females also had the strongest preference for edge habitats among the snake groups. Edge habitats are likely to provide the best opportunities for thermoregulation in the study area because the boundary between "open" and "closed" habitats allows snakes to shuttle between warm, sunny micro climates and cool, shady micro climates with relatively little travel cost. Therefore, the differences in habitat use of gravid females in this oviparous species seem to parallel what has been documented in viviparous species.

It is not unusual to observe behavioural differences between gravid and non-gravid females (e.g., lower frequency of movement, higher frequency of basking: Schwarzkopf and Shine 1991). These differences are most often explained as being a consequence of gravid females having higher thermoregulatory demands than non gravid females (Charland and Gregory 1990, Cobb and Peterson 1991). Alternative explanations could be that the impaired mobility of females when gravid (Seigel et al. 1987, Cooper et al. 1990) forces them to alter their prey, or to rely more heavily on refuges from predators, both of which could result in altered habitat use. The possibility that female black rat snakes alter their prey selection when gravid seems unlikely because their postpartum weight showed evidence of fasting and their movement patterns suggested that they were not foraging. On the other hand, I found evidence that gravid females spend more time concealed and use habitats that provide more cover than non-gravid females or males, which is consistent with the hypothesis that gravid females alter their habitat use in response to higher predation risks. However, it seems unlikely that gravid females use edges for the sole purpose of predator avoidance because retreat sites (e.g., rock crevices, hollows in dead trees) were abundant in other habitat types in the study area. Therefore, the most parsimonious explanation for the stronger preference of edges by gravid female black rat snakes is that edges offer them better thermoregulatory opportunities and that within these edge habitats, gravid females select sites that also offer protection against

predators. To demonstrate formally that the habitat divergence between reproductive females and the other snake groups is linked to thermoregulation, I will need to determine that gravid females thermoregulate differently from males and non-gravid females, and that the difference is related to the habitat they use.

Although "natural" edges were much more abundant than "artificial" edges in the study area, black rat snakes used "artificial" edges and "natural" edges in proportion to their availability. This result indicates that both types of edges seem to fulfil their habitat requirements. From a thermoregulation perspective, it is reasonable to think that both types of edges would allow behavioural adjustments of body temperature at low costs since snakes can easily shuttle between sun and shade in both types of edges. If snakes favour edges because of the better foraging opportunities they provide, then my results tend to suggest that both "natural" and "artificial" edges have higher prey densities than the other habitat types.

My analysis of the seasonal patterns of habitat preference showed that edges were used disproportionately in all months, indicating that they are preferred throughout the active season. However, there was an increase in the use of edge habitats from May to August. This is a pattern opposite to what was reported by Weatherhead and Charland (1985) who found that the preference for edges of black rat snakes was most pronounced early in the season. Several factors could explain the discrepancy between the results of

these two studies. First, the Weatherhead and Charland (1985) study had small sample sizes: seven snakes and 118 characterised snake locations. Second, their study area was much smaller (7 ha) and comprised proportionally more human-made habitats (ca. 20% fields, many buildings) than the present study area (2375 ha, 3% fields, few buildings). If black rat snakes were using edges solely to take advantage of the high density of breeding birds typically found in these habitats, one would expect that the use of edges would be most pronounced during the spring because this is when most nesting occurs. Therefore, my results imply that either the total prey abundance (birds and small mammals combined) actually increases throughout the active season in edges as compared to other habitat types, or that edge habitats are used for purposes other than foraging (all the purposes do not need to be mutually exclusive). Again the most obvious other purpose for the use of edge habitats is thermoregulation.

The results presented in this Chapter have several implications for conservation. Weatherhead and Charland (1985) had hypothesised that the ideal habitat for black rat snakes was a mosaic of field and forest where the scale of fragmentation was sufficiently small that each individual snake could include both forest and habitat edges within its home range. The results I presented here lend support to this hypothesis and extend its implications to more pristine landscapes. The ideal pristine habitat for black rat snakes would also be a mosaic of forest and "open" habitats, but the "open" habitats would

be natural (e.g., marshes, rock outcrops) instead of man-made (e.g., fields). One important aspect to emphasise is that the scale of fragmentation should be sufficiently small that each individual can include forest and forest edges within its home range. Obviously, large-scale land clearing would not be beneficial to black rat snakes because they use habitat edges and not the cleared habitats *per se*. In fact, large-scale land clearing for agriculture has been hypothesised to be the probable cause of the almost complete disappearance of black rat snakes from southwestern Ontario (Prior and Weatherhead 1998).

One of the mechanisms most often invoked to explain how forest fragmentation is detrimental to forest species is the increase in the predation rates near edges (Gates and Gysel 1978, Andrén and Angelstam 1988). Predators identified as being favoured by the increase in edge habitats are often corvids (Moller 1988, 1989, Andrén 1992) or mammals (Small and Hunter 1988). However, it has also been demonstrated that human modifications of the habitat are beneficial to some edge-associated snakes (Henderson and Winstel 1995). In this chapter, I have shown that black rat snakes exhibit a strong preference for edge habitats. Since snakes in general are important nest predators (Robinson et al. 1995), and black rat snakes in particular (Fitch 1963, Stickel et al. 1980), if the increase in edge habitats caused by fragmentation increases rat snake abundance, then black rat snakes can play an important role in the predation dynamics of fragmented ecosystems. Thus, conservation efforts intended to protect black rat snakes by preserving

or creating their preferred edge habitats have the potential to contribute to declines of forest-interior species (mostly birds) that suffer from habitat fragmentation. Clearly, there will be conflicting management issues between black rat snakes and forest birds. Ways to reconcile these conflicting management issues (or at least alleviate their effects) might be suggested by a thorough understanding of the actual role played by black rat snakes in the predation dynamics of fragmented ecosystems.

Table 1-1: Structural variables used in the analysis of habitat selection by Ontario black rat snakes in 1996 and not sampled from 1997 to 1999 with associated mnemonic acronyms and sampling radii (DBH = diameter at breast height).

Acronym	Radius	Variable
DSNAG	30 m	Distance (m) to nearest snag
DBHSNAG	30 m	DBH (cm) of nearest snag (≥30 cm DBH)
DECSNAG	30 m	Decay state (scale from 1 to 7) of nearest snag
DBHOVER	30 m	DBH (cm) of nearest overstory tree
LROCK	30 m	Length (cm) of nearest rock (≥20 cm)
LLOG	30 m	Length (m) of nearest log (≥7.5 cm diameter)
DLOG	30 m	Mean diameter (cm) of nearest log (≥7.5 cm)
NUNDER	5 m	Number of understory trees (<7.5 cm DBH, >2 m height)
%SOIL	2 m	Coverage (%) of bare soil within plot
%HERBS	2 m	Coverage (%) of herbs (non-woody) within plot
HGRDVEG	2 m	Height (m) of ground vegetation (shrubs and herbs)
NWOODY	2 m	Number of woody stems
HCAN	2 m	Height (m) of canopy

Table 1-2: Structural variables used in the analysis of habitat selection by Ontario black rat snakes from 1997 to 1999 with associated mnemonic acronyms and sampling radii (DBH = diameter at breast height).

Acronym	Radius	Variable
DROCK	30 m	Distance (m) to nearest rock (≥20 cm length)
DLOG	30 m	Distance (m) to nearest log (≥7.5 cm diameter)
DOVER	30 m	Distance (m) to nearest overstory tree (≥7.5 cm DBH)
DUNDER	30 m	Distance (m) to nearest understory tree (<7.5 cm DBH, >2 m height)
DEDGE	100 m	Distance (m) to nearest edge
7.5-15	10 m	Number of trees ≥7.5 and <15 cm DBH in plot
15-30	10 m	Number of trees ≥15 and <30 cm DBH in plot
30-45	10 m	Number of trees ≥30 and <45 cm DBH in plot
>45	10 m	Number of trees ≥745 cm DBH in plot
%ROCK	2 m	Coverage (%) of rocks within plot
%LEAF	2 m	Coverage (%) of leaf litter within plot
%LOG	2 m	Coverage (%) of logs within plot
%GRASS	2 m	Coverage (%) of grass within plot
%SHRUB	2 m	Coverage (%) of shrubs within plot
CANCLO	45°	Canopy closure (%) within cone

Table 1-3: Distribution of habitat types for random locations, male locations, non-gravid female locations, and gravid female locations. Percent of column total is shown in parentheses.

Habitat type	Random	Males	Non-gravid females	Gravid females
Artificial edge	19 (11.5)	31 (15.9)	30 (15.8)	22 (27.2)
Natural edge	51 (30.9)	86 (44.1)	83 (43.7)	32 (39.5)
Field	5 (3.0)	2 (1.0)	2 (1.0)	2 (2.4)
Forest	69 (41.8)	74 (37.9)	69 (36.3)	24 (29.6)
Wetland	7 (4.2)	2 (1.0)	6 (3.2)	1 (1.2)
Water body	14 (8.5)	0 (0.0)	0 (0.0)	0 (0.0)
Total	165	195	190	81

Table 1-4: Means (standard errors) of all the variables used in the analysis of habitat selection (defined in Table 1-1 and Table 1-2) for the random locations, the male locations, the non-gravid female locations, and the gravid female locations.

Variable	Random (n = 165)	Males (n = 195)	Non-gravid females (n = 190)	Gravid females (n = 81)
DROCK	5.43 (0.64)	2.45 (0.29)	2.85 (0.28)	2.60 (0.35)
DLOG	5.64 (0.58)	3.22 (0.31)	3.04 (0.35)	2.23 (0.29)
DOVER	4.84 (0.54)	2.26 (0.19)	2.70 (0.24)	1.56 (0.26)
DUNDER	3.93 (0.55)	1.83 (0.24)	1.84 (0.21)	1.23 (0.15)
DEDGE	25.84 (4.37)	17.42 (3.41)	17.46 (3.12)	18.68 (3.39)
7.5-15	12.47 (0.82)	10.81 (0.64)	10.24 (0.66)	7.91 (0.87)
15-30	5.61 (0.42)	4.71 (0.27)	4.62 (0.31)	3.22 (0.37)
30-45	1.35 (0.12)	1.42 (0.11)	1.36 (0.12)	1.49 (0.17)
>45	0.36 (0.07)	0.32 (0.05)	0.40 (0.05)	0.78 (0.10)
%ROCK	8.33 (1.14)	16.63 (1.51)	17.81 (1.44)	11.99 (1.62)
%LEAF	27.67 (1.85)	20.61 (1.43)	23.21 (1.57)	24.22 (2.30)
%LOG	7.02 (0.97)	11.32 (0.81)	11.94 (0.89)	14.31 (1.61)
%GRASS	22.70 (1.90)	16.64 (1.29)	16.09 (1.36)	15.01 (1.98)
%SHRUB	16.62 (1.45)	20.34 (1.46)	22.26 (1.63)	26.25 (2.26)
CANCLO	55.59 (2.96)	53.53 (2.22)	49.53 (2.40)	56.67 (3.12)

Table 1-5: Distances between the four group centroids in the discriminant space (*F* statistic with 15 and 613 degrees of freedom) and their statistical significance.

Group	Random	Males	Non-gravid females
Males	1.40 (11.47)		
	$p < 0.001$		
Non-gravid females	1.25 (8.97)	0.44 (1.22)	
	$p < 0.001$	$p = 0.25$	
Gravid females	2.06 (15.08)	1.10 (4.54)	1.09 (4.39)
	$p < 0.001$	$p < 0.001$	$p < 0.001$

Table 1-6: Summary statistics for the three discriminant functions and their pooled within-group correlations (*r*) with the discriminating variables.

Statistic	Function 1	Function 2	Function 3
Eigenvalue	0.4423	0.0754	0.0227
χ^2 test	$\chi^2_{(45)} = 283.3, p < 0.001$	$\chi^2_{(28)} = 59.0, p < 0.001$	$\chi^2_{(13)} = 13.9, p = 0.38$
% variance	81.7	13.9	4.2
r DROCK	0.28	0.37	-0.01
r DLOG	0.33	0.04	0.53
r DOVER	0.47	0.02	-0.15
r DUNDER	0.38	0.03	-0.01
r DEDGE	0.30	-0.26	-0.08
r 7.5-15	0.15	-0.28	0.07
r 15-30	0.13	-0.35	0.06
r 30-45	-0.04	0.07	0.13
r >45	-0.18	0.59	-0.07
r %ROCK	-0.21	-0.29	-0.08
r %LEAF	0.04	0.19	-0.13
r %LOG	-0.34	-0.15	-0.14
r %GRASS	0.10	0.05	0.32
r %SHRUB	-0.30	0.24	-0.19
r CANCLO	-0.15	0.11	0.29

Table 1-7: Number of locations (percent of total for column) in "artificial" edges and "natural" edges in both the random locations and the snake locations.

Locations	Artificial edges	Natural edges	Total
Random	19 (18.6)	51 (20.2)	70 (19.8)
Snake	83 (81.4)	201 (79.8)	284 (80.2)
Total	102	252	354

Table 1-8: Number of snake locations (percent of total for column) in edge habitats and other habitat types for the four months of the primary active season.

Month	May	June	July	August	Total
Edge	59 (51.8)	84 (59.6)	68 (66.0)	63 (70.8)	274 (61.3)
Other	55 (48.2)	57 (40.4)	35 (34.0)	26 (29.2)	173 (38.7)
Total	114	141	103	89	447

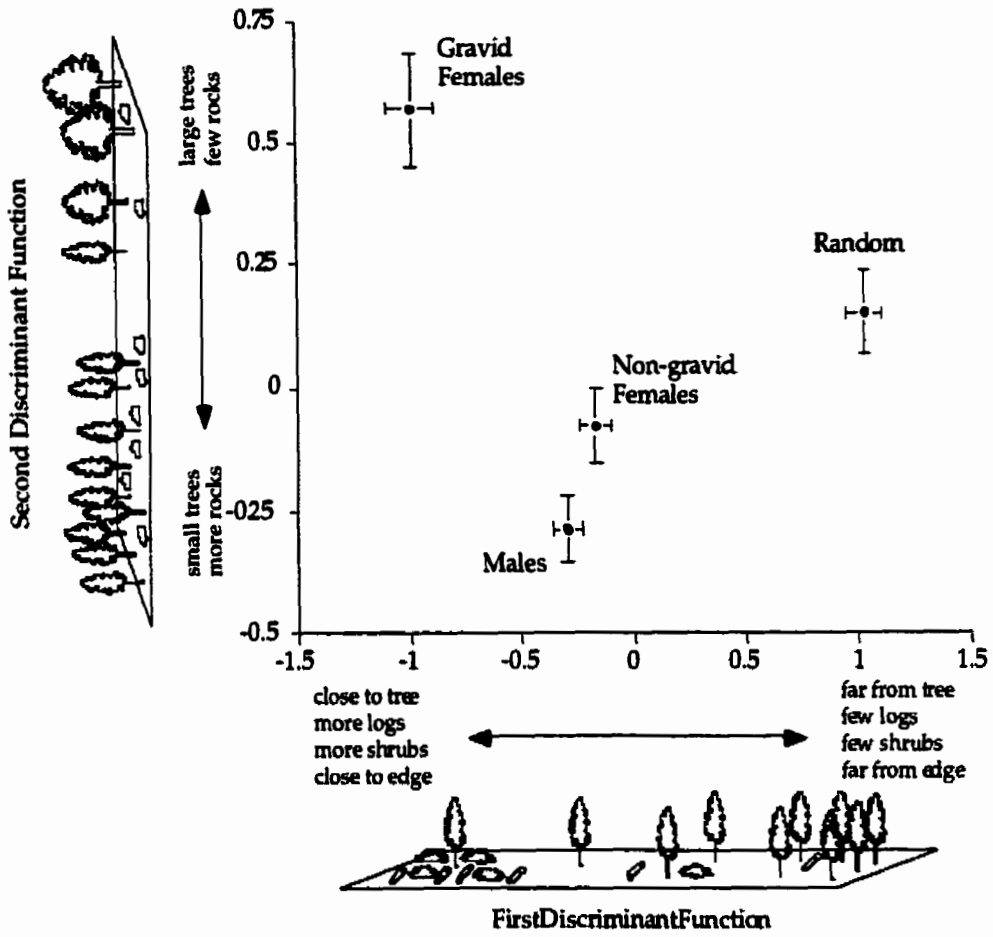

Fig. 1-1: Positions and standard errors of the group centroids of random locations and locations of gravid female, non-gravid female, and male black rat snakes on the two significant discriminant axes with pictorial interpretation of associated habitat gradients in the analysis of habitat use by radio-implanted black rat snakes in Ontario from 1996 to 1999.

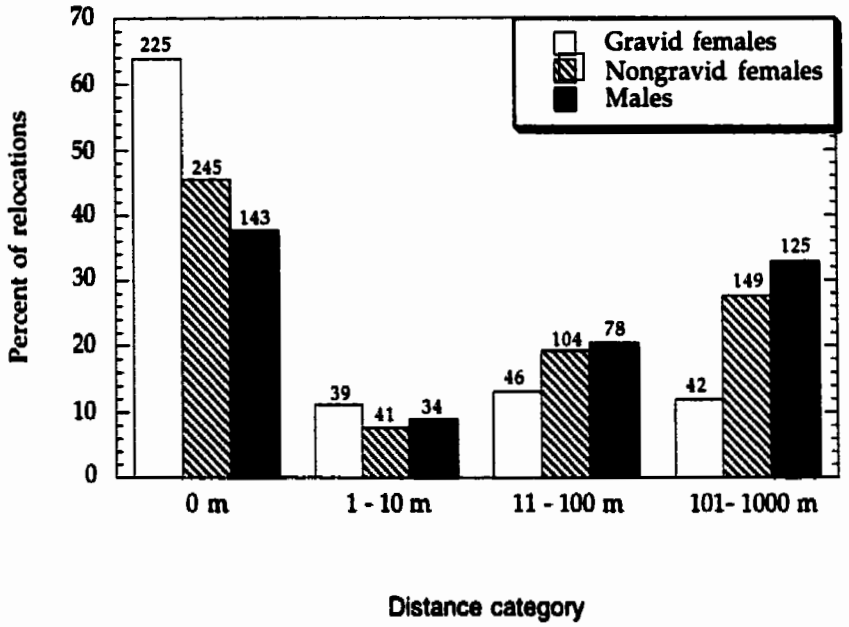

Fig. 1-2: Distance categories moved between successive relocations for radio-implanted gravid female, non-gravid female, and male black rat snakes in Ontario from 1996 to 1999. Only relocations from the time period when females carry their eggs were included and sample sizes are shown above each bar.

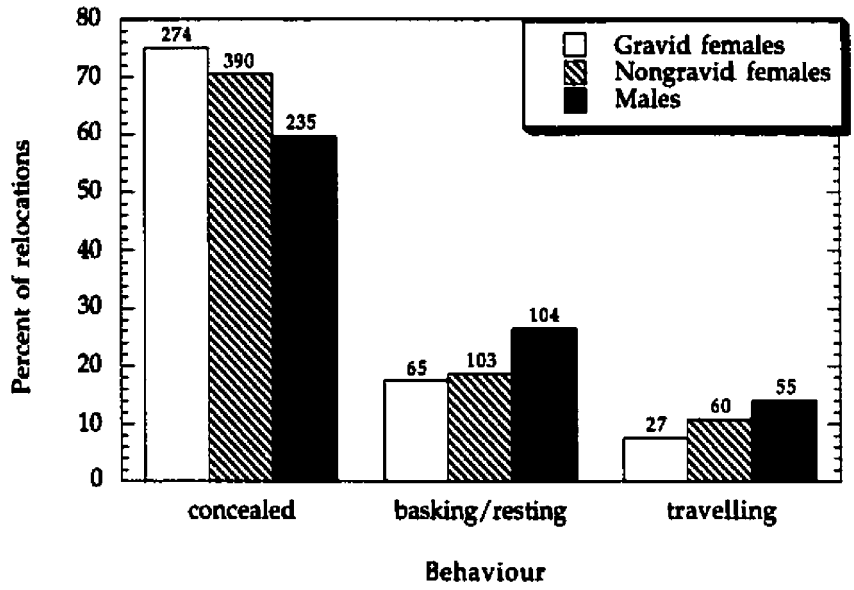

Fig. 1-3: Behaviour exhibited at relocation for radio-implanted gravid female, non-gravid female, and male black rat snakes in Ontario from 1996 to 1999. Only relocations from the time period when females carry their eggs were included and sample sizes are shown above each bar.

CHAPTER TWO

Thermoregulation in black rat snakes (*Elaphe obsoleta obsoleta*)

This chapter formed the basis for the following publication:

Blouin-Demers, G. and P. J. Weatherhead. In press. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. Ecology.

Introduction

All physiological processes are temperature dependent. Thus, because squamate reptiles are ectothermic, variation in their body temperature (T_b) affects their developmental, physiological, and behavioural processes (Schieffelin and De Queiroz 1991, Keogh and DeSerto 1994). Because virtually all aspects of snake ecology are affected by T_b, T_b ultimately has a significant impact on fitness (Huey and Kingsolver 1989). T_b regulation is achieved by adjusting microhabitat selection and timing of activity (Patterson and Davies 1982, Huey et al. 1989, Krohmer 1989, Grant 1990) and, thus, thermoregulation is probably the single most important proximate factor affecting habitat selection and timing of activity of terrestrial reptiles (Grant 1990, Reinert 1993). Although temperature relationships are expected to have an important impact on habitat use by temperate-zone snakes, our understanding of the influence of thermoregulatory requirements on habitat use is still very limited (Peterson et al. 1993). My first general goal in this chapter was to investigate the thermal ecology of black rat snakes to understand the link between thermoregulation and habitat use in this species.

Reproductive state may influence thermoregulation and habitat use by females. Gravid females of many viviparous species have been shown to thermoregulate differently from non-gravid females and males (e.g., Charland and Gregory 1990, Cobb and Peterson 1991, Brown and Weatherhead

2000), and the difference in thermal preference appears to be associated with altered habitat preferences (Reinert 1984a,b, Reinert and Zappalorti 1988). Shine and Madsen (1996) suggested that oviparous snake species should behave similarly, but thus far such behaviour has not been formally documented. In Chapter One I showed that habitat use by gravid female black rat snakes (an oviparous species) was significantly different from habitat use by non-gravid females and males. Gravid females had the strongest preference for habitat edges among the three groups of snakes and I proposed that this was a function of their higher thermoregulatory needs. My first objective in this chapter was to test the prediction that the thermoregulatory behaviour of gravid female black rat snakes differs from that of non-gravid females and males. This difference should be most pronounced early in the season when females are actually carrying eggs (until the first two weeks of July).

In addition to the relevance of this Chapter to understanding habitat selection in black rat snakes, I also used my observations to provide insight into the thermal ecology of snakes in general. Some reptiles are precise thermoregulators and are only active under a narrow range of T_b (Adolph 1990). Other species are thermal conformers and are active under a broader range of T_b (Ruibal and Philibosian 1970, Moore 1978, Hertz 1992a). Difference between species in the costs and benefits of thermoregulation are assumed to account for this variation (Huey and Slatkin 1976). The most obvious cost is

time, because time spent thermoregulating, or waiting for conditions that allow thermoregulation, is not available for other activities (Reinert 1984a,b, Reinert and Zappalorti 1988). The time an ectotherm requires to thermoregulate precisely (thermoregulation cost) depends on the biophysical environment, which includes factors such as the spatial distribution of operative environmental temperatures (T_e) in the habitat (Withers and Campbell 1985), competition (Labra 1995), and predation risk (Huey 1982). The main benefit of thermoregulation is physiological, because physiological processes are T_b dependent and occur optimally under a narrow range of T_b (Peterson et al. 1993). Under the cost-benefit model (Huey and Slatkin 1976), thermoconformity is expected in circumstances where thermoregulatory behaviour would impose such high costs to the organism that precise thermoregulation is not worthwhile (Withers and Campbell 1985), such as in climatic extremes. Very few studies have been conducted at climatic extremes, reflecting the bias for the study of warm-temperate species (Shine and Madsen 1996). Hence, we are faced with a paucity of information to test the broad applicability of this general thermoregulation model. My second general goal in this chapter was to use my data on the thermoregulatory behaviour of black rat snakes to broaden our understanding of the factors that favour thermoregulation versus thermoconformity and thus refine the cost-benefit thermoregulation model.

Black rat snakes in eastern Ontario are at the northern extreme of their range, and are close to the northern limit of snake distributions in eastern North America (Conant and Collins 1991), and thus provide a good opportunity to test the cost-benefit model of thermoregulation. Black rat snakes in Ontario experience cool temperatures and their activity is temporally constrained (early May to early October). They also occur in mostly forested landscapes that reduce the availability of habitats in which basking is possible. My second objective was to quantify the thermal quality (from an ectotherm's perspective) of the snake's environment by assessing the quality of the different habitats available to black rat snakes. The thermal quality of a habitat has been defined as the extent to which the habitat allows an ectotherm to achieve T_bs within their preferred body temperature range (Hertz et al. 1993). Given the numerous thermal constraints faced by black rat snakes, it may be too costly for them to thermoregulate precisely in this environment. Thus, my last objective was to test the prediction that black rat snakes in Ontario are thermoconformers.

Materials and Methods

Study area and study species

I conducted this study from 1997 to 1999 at the Queen's University Biological Station (QUBS), approximately 40 km north of Kingston, Ontario. The study site is close to the geographic centre of the Frontenac Axis

population of black rat snakes in eastern Ontario and northern New York state (Fig. 1 in Blouin-Demers et al. 2000b). The study area was approximately 9.5 km x 2.5 km and was mostly covered by second-growth deciduous forest. However, lakes, beaver ponds and other wetlands, and rock outcrops provided natural gaps and associated edge habitats in the otherwise continuous forest. Artificial gaps and edges were created by small hayfields and some secondary dirt roads in the study area.

Radio-telemetry

I used two different techniques to capture black rat snakes. The majority of the snakes were captured during spring emergence by building fences fitted with funnel traps around communal hibernacula (Blouin-Demers et al. 2000b). I also captured all snakes encountered opportunistically during field work throughout the active season. All snakes were brought back to the laboratory where they were permanently marked by PIT-tagging (Anitech Identification Systems Inc., Markham, Ontario), sexed by probing the cloaca for the presence of hemipenes with a lubricated cloacal probe, measured for snout-vent length (SVL) to the nearest mm with a flexible measuring tape, and weighed to the nearest g with a calibrated spring scale. From all the animals captured, I selected a subset to be implanted with temperature-sensitive radio-transmitters (Model SI-2T, Holohil Systems Inc., Carp, Ontario, 8.6 g, 20 months battery life at 20°C). My choice of individuals was based on sex and size. Snakes had to be large enough to bear the

transmitter (maximum ratio of transmitter mass : body mass = 0.025 : 1) and because females reproduce every second or third year on average (G. Blouin-Demers, unpublished data), I had to implant more females than males to obtain an adequate sample of gravid females. I used isoflurane delivered via a precision vaporiser to anaesthetise the snakes and then sterile surgical techniques to implant the radio-transmitters in the body cavity and the antennae just underneath the epidermis. The surgical technique was a modification and improvement of the procedure presented by Reinert and Cundall (1982). However, the anaesthesia procedures had to be developed for this study because little information regarding reptilian anaesthesia is available in the literature. Also, preliminary trials indicated that black rat snakes did not respond well to the few procedures that were published. In Appendix One I present the detailed anaesthesia and surgical procedures I used.

After release, I located the snakes on average every 48 hrs using a telemetry receiver (TRX-2000S, Wildlife Materials Inc., Carbondale, Illinois) and a directional three-element antenna (F173-3FB, Wildlife Materials Inc., Carbondale, Illinois), from the snakes' emergence in late April to early May until they re-entered their hibernacula in late September to early October. In Ontario, black rat snakes have a continuous hibernation period that they spend inactive in communal hibernacula (Weatherhead 1989). Therefore, during hibernation snakes were only checked every 3 weeks on average to

determine their body temperature from the pulse rate of the transmitter (see below). For the purpose of this study, I considered the active season of black rat snakes to be from 1 May to 30 September based on the average date of 6 May for 801 exits from hibernation by 551 individuals (Blouin-Demers et al. 2000b), and on the average date of 4 October for 59 entries into hibernation by 41 radio-implanted snakes recorded during the present study. From May 1997 to November 1999, I followed 17 males and 36 females for periods ranging from 1 to 30 months. Because females do not reproduce every year, eight females were followed both when they were gravid and non-gravid. Thus, I have data from 25 individual females when non-gravid and from 19 individual females when gravid. Twenty-three snakes (nine males and 14 females) were followed in multiple years and therefore I have data for 79 "snake years" (25 "male years", 33 "non-gravid female years", and 21 "gravid female years").

The radio-transmitters I used were temperature-sensitive (i.e., the pulse rate of the radio-transmitter was proportional to temperature). Calibration curves were supplied by the manufacturer for each radio-transmitter (based on pulse rates determined from 0°C to 40°C in 5°C increments), but I checked their accuracy using a water bath and a mercury thermometer. My own calibrations were always within 0.5°C of the manufacturer's, and I therefore used the latter calibrations in my calculations. When removing radio-transmitters for battery replacement, I determined

whether any drift had occurred in the calibration curve. No significant drift (i.e., a shift of ≥ 0.5°C in estimated temperature at a given pulse rate) was ever recorded. I used polynomial regressions (including all terms up to degree 4) and the eight calibration points for each radio-transmitter to derive an equation to predict temperature based on pulse rate. All calibration equations provided a very high degree of fit (all R^2's ≥ 0.9999). This proved to be a more accurate and much more efficient way to determine temperature based on the pulse rate of the radio-transmitter than using the calibration curve supplied by the manufacturer to read the temperature for each pulse rate recorded.

Body temperatures of black rat snakes were obtained in two ways. First, each time a snake was located (every 48 hrs during the active season, every 3 weeks during hibernation), I obtained the pulse rate of the transmitter by recording the duration of 10 pulse intervals with a stopwatch and then dividing by 10 to obtain the pulse rate of the transmitter. I then used the calibration equation of the transmitter to determine the T_b of the individual. Second, each season I used two automated radio-telemetry data loggers (SRX 400, Lotek Engineering Inc., Newmarket, Ontario) powered by marine deep-cycle 12 V batteries to record daily body temperature profiles of black rat snakes from the emergence of the first radio-implanted individual (April) to the entry into hibernation of the last individual (October). I entered all the calibration equations in both loggers and then programmed the loggers to scan the frequency of each radio-transmitter every 10 minutes. Each time a

snake was within transmission range (the signal of the radio-transmitter was clearly distinguished against the background electrostatic noise), the loggers calculated the pulse rate as the average duration of three pulse intervals, computed the corresponding temperature from the calibration equation of that transmitter, and stored the date, time, transmitter frequency, and temperature. The built-in memory of the loggers allowed seven days of data to be recorded on average (depending on the number of snakes that were within range) before the data needed to be downloaded to a computer in the form of a spreadsheet. Because black rat snakes are wide-ranging and the transmission range was limited to approximately 500 m (depending on the snake's position: e.g., transmission range was greater when snakes were in trees), it was not possible to have complete body temperature profiles for all individuals. However, I regularly moved the two loggers in the study area to maximise the number of snakes that were within transmission range at any given time.

Thermal preference

In all my analyses of thermoregulation, I used the terminology and symbols introduced by Hertz et al. (1993) and subsequently followed by most researchers. The first step in studying thermoregulation is to identify the preferred body temperature range (T_{set}) of the species of interest. T_{set} should be measured in an environment where there are no thermoregulatory costs (Huey and Slatkin 1976, Hertz et al. 1993). Hence, I measured T_{set} using a

thermal gradient chamber in the laboratory. The chamber was constructed using a large rectangular plywood box (250 cm x 60 cm x 60 cm). A fluorescent light over the chamber provided constant and homogeneous illumination. I established a thermal gradient by placing one end of the chamber over a coil of tubing through which cold water was constantly circulating, and the other end over a heating pad, producing a smooth gradient from 15 to 40 °C. I introduced post-absorptive (fasted for seven days) radio-implanted rat snakes individually in the chamber and gave them 24 hrs to acclimatise to the setting. Then I recorded the body temperature they selected in the gradient every 10 min for 24 hrs using one of the telemetry data loggers. The bounds of the central 50% of observed T_bs for each individual were used to determine T_{set} (Hertz et al. 1993, Christian and Weavers 1996).

Environmental operative temperatures

The range of possible T_bs available to an ectotherm in the field is referred to as the operative environmental temperature (T_e). In a classic experiment involving water-filled beer cans as physical models of ectotherms, Heath (1964) showed that T_e often differs from ambient temperatures (T_a) and that studies using T_a as a measure of available T_b can thus lead to erroneous conclusions about thermoregulation. T_e can be determined using complex mathematical models that integrate all the heat exchange parameters to predict T_b for a given species under a fixed set of environmental conditions

(Scott et al. 1982, Bakken 1992). A simpler approach is to install in the field a series of physical models that have the same thermal characteristics as the species of interest. These physical models are used as "T_e thermometers" and allow one to quantify over time and space the T_b range an ectotherm could potentially achieve in the field. This gives the null hypothesis of thermoregulation (i.e., thermoconformity: Hertz 1992b, Hertz et al. 1993) to which one compares the actual field T_b of the study animals to investigate thermoregulatory behaviour. I used a combination of these two approaches in the present study.

I constructed two models of black rat snakes using copper pipes. The models were 40 cm long and had a diameter of 4 cm. I painted the models with glossy black metal paint to approximate the reflectance of black rat snakes (Peterson et al. 1993). I suspended a thermocouple in the centre of each model with stiff metal wire, filled the models with water, and then sealed both ends of each model with a plastic cap and silicone. I calibrated the models using two fresh carcasses of rat snakes found dead on the road. The carcasses were at each extreme of the range of masses of snakes with transmitters: one weighed 400 g and the other 1200 g. I placed the two carcasses beside one of the copper models on bare ground just before dawn on three sunny days in August 1998 and recorded the temperatures in the carcasses and the model every 20 minutes until dusk using one of the

telemetry data loggers and a radio-transmitter placed in each carcass and in the copper model.

To record environmental operative temperatures, one model was placed on bare ground in the open and its thermocouple was permanently connected to the meteorological station at QUBS where model temperature, air temperature 1.5 m above ground in a Stevenson screen, wind speed at 5 m above ground, and solar radiation at 2 m above ground were recorded every minute and averaged for each hour. The thermocouple of the other model was connected to a miniature temperature data logger (HOBO Temp, ONSET Computer Corporation, Pocasset, Massachusetts) that recorded the model's internal temperature every 10 min, which I then averaged for each hour. I alternately placed this second model in at least four locations in a given habitat (see below) for a total duration of 3 to 6 weeks and then moved it to another habitat.

My aim was to measure operative environmental temperatures in all the habitats available to black rat snakes. The different habitats in the study area could be classified as forest, rock outcrop, field, wetland, and water body (Chapter One). I measured T_es by placing the fixed model on bare ground in the open and the portable model snake in forested habitat, in hay field habitat, and in rock outcrop habitat. When positioning the model in the different habitats, I was sensitive to the natural history of black rat snakes and I tried to select microhabitats that were available to snakes. I always placed the model

on the ground, except in forests where the model was also placed on tree branches. Because black rat snakes are terrestrial (Chapter One), I did not measure T_e in water. However, rat snakes are found near the edges of wetlands (primarily marshes with sedges, *Carex*, and cattails, *Thypha*). Because these wetlands are structurally similar to fields, I considered the T_es for wetlands to be the same as for fields. Finally, there were a number of retreat sites (rock piles, crevices in rock outcrops, large logs, old barns, old machinery, inside snags) buffered from outside variation in microclimate that were used by all black rat snakes and that could not be adequately classified in any of the above categories. Therefore, I also placed the portable model in two representative and common retreat sites (under flat rocks on rock outcrops and in barns) to measure the T_es available to black rat snakes in such retreats.

To determine the T_e available in all habitats at all times I needed to derive multiple regression equations to predict model temperature in a given habitat based on the four microclimatic variables. I used the MAXR procedure of SAS (SAS Institute 1990) to find the combination of climatic variables that best predicted model temperature (maximised the R^2) in each of the habitats. For four variables, the MAXR procedure finds the best one-variable model, the best two-variable model, the best three-variable model, and the best four-variable model. Among these four models for each habitat, I chose the one that included the most significant variables based on their Type III sum of

squares because I had no *a priori* reason to favour a particular ordering of the variables. I used the equation of each of the selected models to generate T_es for each habitat for the duration of the study. To obtain a measure of the average operative temperature of the habitat available to black rat snakes, I calculated a mean T_e as the average of the T_es of all the habitats for each hour. This method of calculating mean T_e assumes that each snake had access to all habitats in the study area. This assumption seems reasonable because (1) black rat snakes can travel long distances in a short time period (e.g., ≈ 5000 m in two days: G. Blouin-Demers, unpublished data), (2) the habitat patches in the study area are interspersed and small in comparison to the size of the snakes' home ranges, allowing several habitats to be encompassed within a home range (Weatherhead and Charland 1985, Weatherhead and Hoysak 1989), and (3) black rat snakes are not territorial and have broadly overlapping home ranges and thus, are apparently free to choose the best available habitat (Weatherhead and Hoysak 1989).

Extent of thermoregulation

Several methods have been designed to describe the thermal ecology of a species. Thermoregulation indices developed in recent years compare the extent to which a species actually experiences T_bs within its T_{set} (the "accuracy" of T_b) to the extent to which the habitat in which the species lives allows T_bs within the T_{set} to be reached (the "thermal quality" of the habitat).

Following the protocols established by Hertz et al. (1993), I measured the accuracy of T_b as the mean of the deviations of field active T_bs from T_{set} (individual deviation = d_b). If T_b is below the preferred range, then d_b is the difference between the lower bound of T_{set} and T_b and if T_b is above T_{set}, d_b is the difference between T_b and the upper bound of T_{set}. Similarly, I measured the thermal quality of each habitat by the mean of the deviations of T_es from T_{set} (individual deviation = d_e) in each habitat. If T_e is below the set point range, d_e is the difference between the lower bound of T_{set} and T_e and if T_e is above T_{set}, d_e is the difference between T_e and the upper bound of T_{set}.

In Chapter One I showed that black rat snakes preferentially use the edges between forest and open habitats such as rock outcrops or fields. To evaluate the thermal quality of edges, I assumed that snakes in an edge had access to the habitats on either side of the edge at virtually no cost. I defined edges as the habitat within 15 m of the boundary between forest and any open habitat. Thus, because virtually no travel time is required for a snake to shuttle between the two habitats along an edge (with maximum body lengths approaching 2 m a snake could be in both habitats simultaneously), the assumption of no travel costs seemed reasonable. Also, because T_es exceeded the upper bound of T_{set} in only 1.5% of the observations in the forest (see Results), I considered that forested habitats always provided a refuge from high temperatures. Therefore, to calculate d_e in edges, I was only interested in instances where the lower bound of T_{set} could not be reached in the forest or

in the open habitat because even when T_e exceeded the upper bound of T_{set} in the open habitat, snakes could use the forested habitat to cool down. Hence, I assigned d_e a value > 0 only when T_e in both habitats was lower than the lower bound of T_{set}. I defined d_e in edges as the minimum deviation from the lower bound of T_{set} in the forest or in the open habitat. Using this method, I calculated d_es for the edge between forest and rock outcrop and the edge between forest and field/wetland. Snakes could also use the edge of water bodies. To calculate d_es for the edge between forest and water bodies I used the above method using T_es for the model snake on open bare ground. Open bare ground is structurally very similar to the open habitats available at the edge of water bodies in the study area.

From the measures of the accuracy of T_b (= d_b) and of the thermal quality of habitats (= d_e), Hertz et al. (1993) derived an index of the effectiveness of thermoregulation (E) that they used to compare the thermoregulatory behaviour of different populations or of different species. Brown and Weatherhead (2000) were the first researchers to use these indices to compare the thermoregulatory behaviour of different segments or different individuals within segments of a population and I follow their approach here. E is calculated as $E = 1 - (\bar{d}_b / \bar{d}_e)$. When animals do not thermoregulate and select microhabitats randomly with respect to T_e, d_b and d_e will be similar and E will tend towards 0. On the other hand, if animals thermoregulate very carefully, d_b will be much smaller than d_e and E will tend towards 1. Negative

values of E indicate that animals actually use habitats with T_es within T_{set} less than their availability. When calculating E, I averaged the d_es for all habitats each hour to obtain a measure of the average thermal quality of the habitat available to black rat snakes. An additional index of thermoregulation (Ex) that determines the extent to which animals exploit the thermal environment was developed by Christian and Weavers (1996). They defined Ex as the amount of time an animal spends within its T_{set}, expressed as a percentage of the time when it was possible for the animal to do so (as indicated by the T_es). Therefore, I calculated Ex as the proportion of T_bs that fell within T_{set} for times when the d_e of at least one of the habitats was equal to zero. Following Brown and Weatherhead (2000), I also modified this index slightly to calculate the proportions of T_bs that fell below and above T_{set} when the d_e of at least one of the habitats was equal to zero. I used these different indices of thermoregulation in this study because the question of how carefully an ectotherm regulates its T_b is actually a suite of questions that require different methods to answer (Hertz et al. 1993). For example, d_b was designed to quantify the accuracy of T_b (i.e., how much T_bs deviate from T_{set} on average), and similarly d_e quantifies the thermal quality of a habitat by providing a measure of how much T_es deviate from T_{set} on average. E was designed to measure the effectiveness of thermoregulation by quantifying (and standardising) how much departure there was from perfect thermoconformity (i.e., $d_e = d_b$), and Ex was designed to measure the extent to

which ectotherms exploit the available opportunities for thermoregulation, but without taking into account the behaviour of the species during times when T_es within T_{set} are not available. Each index therefore answers a slightly different question and, used together, the indices provide an accurate picture of a species' thermoregulatory behaviour.

Statistical analyses

I divided the snakes into three groups based on their reproductive status: males, non-gravid females, and gravid females. The reproductive state of females was assessed in June by externally palpating the oviducts for the presence of eggs and confirmed in July by nesting activity. Because female black rat snakes do not reproduce every year, some individuals changed groups from one year to the next. The reproductive status of individuals was entered as a factor in two-way analyses of variance (ANOVA). Series of T_bs recorded from a single individual are not independent. Therefore, all analyses were performed on data (T_b, E, or Ex) averaged for each individual over the period appropriate for the specific analysis (year, month, hour), thereby circumventing the pseudo-replication problem (Shine and Madsen 1996, Stevens 1996). Some females changed reproductive status from one year to the next (gravid vs. non-gravid). Thus, I considered T_bs measured for an individual in different years independent. For most individual snakes, I did not have complete T_b data for three main reasons: (1) snakes regularly moved out of the data loggers' range, (2) some snakes were killed by predators before

the termination of the study, (3) radio-transmitters failed unexpectedly. Therefore, it was not practical to use repeated measure analyses on individuals to analyse my data.

The analyses were conducted on JMP Version 3.2 (SAS Institute 1997), SPSS Version 6.1 (SPSS 1995), and a mainframe version of SAS (SAS Institute 1990) on a Macintosh desktop computer. I inspected Box plots to determine if the assumptions of normality and homogeneity of variance were upheld. I detected no significant violations of these assumptions. Significance of statistical tests was accepted at $\alpha = 0.05$, but marginally non-significant results are discussed when deemed important. All means are reported ± one standard error unless otherwise stated.

Results

From April 1997 to November 1999, I recorded 150,368 T_bs from the 53 individuals (17 males and 36 females) I followed. Of the 150,368 T_bs, 3,786 (2.5%) were recorded from the pulse rate of the transmitter when I located the snakes and the remaining 146,582 (97.5%) were recorded on the two automated telemetry data loggers. I reduced these body temperatures to 34,211 hourly mean T_bs that I used as the basis for all analyses. Of these hourly mean T_bs, 29,722 (86.9%) were from the active season (1 May to 30 September) and 4,489 (13.1%) were from the hibernation period. Of the T_bs recorded during

the active season, 17,086 (57.5%) were recorded during the day (600h – 1800h) and the remaining 12,636 (42.5%) during the night.

Thermal preference

In total, nine males, 21 non-gravid females, and 11 gravid females were placed in the thermal gradient. For six females, I measured T_{set} both when they were gravid and when they were not gravid. For each individual, I calculated the mean T_b in the gradient and the 75% and 25% quartiles (Hertz et al. 1993). Averaged across all individuals, the mean body temperature selected in the thermal gradient was 28.1°C and the mean 75% and 25% quartiles were 29.8°C and 26.5°C respectively (Table 2-1). One way ANOVA's revealed no significant differences among males, non-gravid females, and gravid females for any of these parameters (mean T_b $F_{(2,38)} = 0.288$, $p = 0.75$; 75% quartile $F_{(2,38)} = 0.050$, $p = 0.95$; 25% quartile $F_{(2,38)} = 0.523$, $p = 0.60$). For the six females tested both when gravid and non-gravid, I contrasted the three thermal selection parameters using paired t-tests. For each parameter, there was no significant difference between reproductive states (mean T_b paired $t_{(5)} = 0.012$, $p = 0.99$; 75% quartile paired $t_{(5)} = 0.030$, $p = 0.98$; 25% quartile paired $t_{(5)} = -0.162$, $p = 0.88$).

To determine if the snakes preferred the same body temperatures in the field as in the laboratory when conditions in the field allowed, I calculated the mean T_b during the active season in the field by each individual and its 75% and 25% quartiles. I restricted these calculations to periods when the T_es

in the different habitats available to black rat snakes indicated that the lower bound of T_{set} (26.5°C) could have been reached in at least one habitat (see Thermal exploitation below). Averaged across all individuals, the mean body temperature in the field during periods where it was possible to have T_bs within T_{set} was 25.2°C, and the mean 75% and 25% quartiles were 28.7°C and 22.1°C, respectively. Therefore, even when conditions in the field allowed black rat snakes to attain T_bs in their preferred range, their mean T_bs, 75% quartiles, and 25% quartiles were lower than those selected in the laboratory thermal gradient (28.1°C, 29.8°C and 26.5°C, respectively). Differences between these laboratory and field temperatures were highly significant (t-tests, all $t_{(90)}$s > 2.53, all ps < 0.01).

Environmental operative temperatures

The temperatures of the 1200 g snake carcass and the copper snake model were highly correlated ($r = 0.98$, $F_{(1,100)} = 1800.3$, $p < 0.001$) and the mean temperatures of the carcass and of the model were not significantly different (mean difference = -0.17°C, paired $t_{(101)} = -1.07$, $p = 0.285$). The temperature of the 400 g carcass was also highly correlated with the temperature of the snake model ($r = 0.98$, $F_{(1,80)} = 2837.4$, $p < 0.001$), but the difference between the mean temperatures of the 400 g carcass and of the model was significant (mean difference = -0.51°C, paired $t_{(81)} = -4.22$, $p < 0.001$) and indicated that the model tended to slightly overestimate the carcass temperature. However, because the mean difference between the model and carcass temperatures was very small

and approximately the same as the transmitter calibration error (my calibrations vs. the manufacturer's), I deemed it not biologically meaningful. Thus, I assumed the snake models accurately measured the available body temperatures for the size range of black rat snakes I was monitoring.

I derived multiple regression equations to predict model snake temperatures in each habitat based on the four microclimatic variables. The maximum simple correlation (r) between pairs of variables used in the analysis was 0.47 and the highest variance inflation factor for any of the equation terms was 2.5. All equations were highly significant and explained a large proportion of the total variance (mean $R^2 = 0.85$, Table 2-2). I used these equations to calculate hourly T_es from 1 May to 30 September of each year for the different habitats.

Thermal quality of habitats

I calculated monthly mean T_es based on all mean hourly T_es measured during the active season over the three years. Mean T_es increased from their lowest point in May to their peak in July and then decreased in August and September, but mean T_e never exceeded the lower bound of T_{set} in any month (Fig. 2-1), indicating that the habitat inhabited by black rat snakes in Ontario is thermally challenging. Forests had the lowest mean T_e (Table 2-3) and were the coolest habitat for most of the day (Fig. 2-1). In fact, T_e exceeded the upper limit of T_{set} (29.8°C) in only 166 of 10,719 observations (1.5%) in the

forest. Therefore, I considered that forests offered rat snakes a permanent refuge from high temperatures. I used the mean d_e calculated for the entire active season to measure the average thermal quality of the habitats available to black rat snakes. Mean d_es varied from 12.0°C to 5.5°C for the different habitats. Mean d_es were highest for the open habitats (field/wetlands, bare ground, and rock outcrops), intermediate for forests, and lowest for the two retreat sites (barns and under rocks) and for the three types of edges (Table 2-3).

Body temperatures

I calculated mean monthly T_bs for each individual in each year based on the hourly mean T_bs and then averaged these individual means for each month of the year. Mean monthly T_bs were at their minimum towards the end of hibernation in March (5.6°C), increased at the start of the active season to reach their peak in June (24.2°C) and July (25.4°C), and then gradually decreased from August to March (Fig. 2-2). The distribution of mean hourly T_bs and T_es during the active season indicated that black rat snakes avoided temperature extremes and selected habitats that allowed them to maintain T_bs above the mean T_e (Fig. 2-3). T_bs were within T_{set} 17.5% of the time whereas mean T_e for all the habitats fell within T_{set} only 8.3% of the time.

I averaged hourly T_bs for each individual for each hour of the day in each year. I similarly calculated mean maximum T_e and mean minimum T_e

for each hour. A plot of mean T_b, mean maximum T_e, and mean minimum T_e through the day revealed that black rat snakes were as warm as their environment allowed at night between approximately 2200h and 600h, but that they did not fully use the opportunities to reach T_bs within the bounds of T_{set} during the day (Fig. 2-4). When I considered the whole active season, the mean hourly T_bs of black rat snakes never reached the lower bound of T_{set} during the day, although it would have been possible to have T_bs within T_{set} between approximately 800h and 1900h daily. Thus, black rat snakes seemed to exploit their thermal environment maximally at night but not during the day. Even if I only consider the warmest month (July), on average black rat snakes maintained T_bs within T_{set} between 1100h and 1800h even though values of T_e indicated that it would have been possible to have T_b within T_{set} between 700h and 2100h (Fig. 2-4).

I also used the mean monthly T_bs for each individual in each year to determine if T_bs differed between the three sex/reproductive groups, and if there was a difference, if this difference varied seasonally. I used mean T_bs as the response variable in a two-way ANCOVA where mean monthly T_e, sex/reproductive group, month, and the interaction between group and month were entered as predictor variables. I entered mean monthly T_e as a factor in the analysis to control for the potential effect of measuring T_bs on different individuals under different climatic conditions. I conducted the same analysis separately for daytime (600h – 1800h) and night-time (1800h –

600h). The interaction terms were not significant, indicating that the relationship between group and T_b did not consistently vary seasonally. Not surprisingly, mean monthly T_e had a significant effect on the T_b maintained by the snakes. The mean T_b of the three reproductive groups were significantly different during the day, but the difference was non-significant at night (Table 2-4). Tukey-Kramer HSD tests indicated that gravid females maintained significantly higher T_bs than males and non-gravid females during the day, but that males and non-gravid females were not significantly different from one another (Fig. 2-5).

Effectiveness of thermoregulation

I calculated a mean d_b for each individual in each year and then calculated E for each individual in each year using the average mean d_e for all the habitats in that year. The mean d_b based on all the individual values was 4.79°C ± 0.22 and the average of the mean d_e for the three years of the study was 8.09°C ± 0.12. The overall average of the individual values of E was 0.409 ± 0.025. Thus, black rat snakes can be referred to as moderately precise thermoregulators given that E would equal 1 if snakes always had T_bs within T_{set} (pure thermoregulation) and would equal 0 if the T_bs were always as far from T_{set} as the T_es (pure thermoconformity).

I used the mean monthly d_bs for each individual in each year to calculate overall mean monthly d_bs. Similarly, I averaged the mean monthly

d_es in each year to obtain overall mean monthly d_es. Plotting overall mean d_bs and overall mean d_es as a function of month indicated these two variable followed a similar pattern. Mean d_bs and mean d_es were highest during May, which was the coldest month, decreased to reach their lowest point in July, which was the warmest month, and then increased again in August and September (Fig. 2-6). The mean d_es were always higher than the mean d_bs, but the difference between d_b and d_e was highest in July and lowest in September (Fig. 2-6). I calculated E for each individual in each month of each year using the mean monthly d_b of the individual and the mean monthly d_e for that month that year. I then averaged all the individual values of E for each month of the active season. As expected from the previous analysis of the seasonal variation in d_b and d_e, E increased in May and June to reach its highest point (0.55) in July and then decreased to its lowest point in September (0.10) (Fig. 2-7).

To see how thermoregulation varied during the course of the day, I also calculated mean hourly d_bs for each individual in each year and then averaged these values for each hour. I similarly averaged mean hourly d_es for each year and then averaged d_es for each hour. Overall, mean d_es were higher than mean d_bs during the course of the day, except from approximately 800h to 1000h where d_es were actually lower than mean d_bs. The difference between d_e and d_b was greatest at night between approximately 2000h and 600h (Fig. 2-6) when the snakes remained as warm as their environment

allowed. I calculated mean hourly *E*s for each individual in each year and then averaged the individual values for each hour. As expected based on the relative fluctuations of d_b and d_e, *E* was highest at night (≈ 0.50) from approximately 2000h to 700h, was actually negative from approximately 800h to 1000h, and was approximately 0.25 for the rest of the day (Fig. 2-7). This pattern is a consequence of black rat snakes selecting the warmest available microhabitats at night (presumably retreat sites), thus producing high values of *E*. However, the snakes then tended to delay their emergence from their nocturnal retreat until approximately 3 hours after the pronounced morning increase in mean T_es, thus producing negative values of *E* in the early morning. Throughout the remainder of the day, T_bs of rat snakes were closer to T_{set} than were mean T_es, but the snakes did not exploit fully the opportunities to increase their T_b to reach the lower bound of T_{set}.

I used two-way ANOVAs to determine if reproductive groups differed in their effectiveness of thermoregulation and if there was a seasonal component to any difference. I calculated *E* for each individual in each month using the mean monthly d_b of an individual and the mean monthly d_e for that month and used these values as my response variable. I entered month, reproductive group, and the interaction of these two factors as predictor variables. I conducted the same analysis separately for the diurnal period (600h – 1800h) and the nocturnal period (1800h – 600h), and the interaction terms were non-significant in both cases. The effect of reproductive group was

significant during the day and not significant at night (Table 2-5). Tukey-Kramer HSD tests indicated that during the day, gravid females had significantly higher E's than males and non-gravid females but that males and non-gravid females were not significantly different from one another (Fig. 2-5).

Thermal exploitation

The proportion of T_bs that fall within T_{set} when climatic conditions make it possible is the thermal exploitation index (Ex) proposed by Christian and Weavers (1996). In addition to Ex, I also calculated the proportion of T_bs that fell below and above T_{set} when T_bs within T_{set} could be reached. Thus, in calculating these three indices of thermoregulation, I restricted the analysis to times where T_{set} could be reached in at least one habitat (i.e., times where d_e in at least one habitat was equal to 0) and looked at the proportion of T_bs that fell below, within, and above T_{set}. Over the three active seasons, I collected T_e data for 10,835 hrs (i.e., T_e data were missing for only 145 hrs over the three years) and d_e was equal to 0 in at least one habitat for 4,825 hrs (44.5% of the time). In total I obtained 29,722 hourly mean T_bs from snakes during the active season over the three years and d_e was equal to 0 in at least one habitat for 15,189 (51.1%) of these observations. I calculated the three thermal exploitation indices for each individual each year. Averaged across all individuals, the proportion of T_bs that fell within T_{set} (the Ex index of

Christian and Weavers, 1996) when it was possible for them to do so was 22.44% ± 1.33. The proportion of T_bs that fell above T_{set} was 17.22% ± 1.34, and the proportion that fell below was 60.34% ± 2.26.

Ex reached its maximum in July (34%) and its lowest point in September (5%) (Fig. 2-8). July was also the month in which the percentage of T_bs below T_{set} reached a minimum, subsequently increasing to a maximum in September (93%) (Fig. 2-8). The percentage of T_bs above T_{set} was highest in June (25%) and decreased to almost 0% in September. During the course of the day, *Ex* was at its minimum around 700h and increased gradually to peak around 2200h, decreasing thereafter (Fig. 2-8). During the early morning hours (300h – 1000h), more than 70% of T_bs fell below T_{set} and T_bs above T_{set} occurred primarily in the afternoon (1200h – 1900h) (Fig. 2-8).

I used three two-way ANOVAs to determine if reproductive groups differed in their thermal exploitation indices ($T_b = T_{set}$, $T_b > T_{set}$, and $T_b < T_{set}$) and if there was a seasonal component to this potential difference. I calculated the three thermal exploitation indices for each individual in each month and used these values as my response variable. I entered month, reproductive group, and the interaction of these two factors as predictor variables. I conducted the same analyses separately for daytime (600h – 1800h) and night-time (1800h – 600h). None of the interaction terms were significant (Table 2-6), indicating that the relationship between reproductive group and these indices did not vary consistently seasonally. For $T_b = T_{set}$, there was no

significant difference between the different reproductive groups during the day or during the night (Table 2-6). For the index $T_b > T_{set}$, the effect of reproductive group was significant during the day and non-significant at night (Table 2-6). Tukey-Kramer HSD tests indicated that during the day gravid females and males had equal proportions of T_bs > T_{set}, but that both groups had significantly more T_bs > T_{set} than non-gravid females (Fig. 2-9). Finally, there was a significant difference between the different reproductive groups in the proportion of $T_b < T_{set}$ during the day, but not at night (Table 2-6). Tukey-Kramer HSD tests again indicated that during the day gravid females had significantly fewer T_bs < T_{set} than non-gravid females, but that males were not significantly different from the other two groups (Fig. 2-9).

Group differences in thermoregulation

One surprising and recurrent result in my analyses of the difference in thermoregulatory behaviour (T_b, E, and Ex) between reproductive groups was the lack of a significant interaction between reproductive groups and months. Gravid female black rat snakes only carry their eggs in the early part of the active season (from early June to mid July). Therefore, I expected that if there was a difference in thermoregulatory behaviour between reproductive groups, this difference should occur during the months prior to egg-laying and there should be no difference thereafter, thus producing a significant and consistent reproductive groups by months interaction. Overall, I found evidence that gravid females thermoregulated more carefully than the other

snakes groups (higher T_bs, higher Es, more T_bs > T_{set} and fewer T_bs < T_{set} when the minimum d_e = 0), but no evidence of a reproductive groups by months interaction.

To investigate this absence of a seasonal effect further, I calculated the least square mean T_bs (corrected for the effect of mean monthly T_e) and the mean of all four thermoregulatory indices separately for each reproductive group in each month based on mean monthly individual values. Plotting the means as a function of month for each group revealed complex interactions between reproductive groups and months. In all cases each factor had more than two levels and the differences between the three reproductive groups varied inconsistently in each month (Fig. 2-10, Fig. 2-11). This variability and inconsistency in the interactions made them non-significant. However, plotting the means as a function of month for each group also revealed that in most cases the difference between gravid females and the other two groups was more pronounced in May and June, the time that females develop and carry their eggs, than for the other months. The mean T_b (adjusted for mean T_e) of gravid females in May and June was ≈ 2°C higher than the mean T_bs of males and non-gravid females, whereas it was only ≈ 1°C higher in July and ≈ 0.5°C higher in August and September (Fig. 2-10). Similarly, the mean E of gravid female in May (≈ 0.5) and June (≈ 0.6) was much higher than the mean E of males and non-gravid females in May (≈ 0.25) and June (≈ 0.3), whereas

the three groups were very similar for the rest of the season (Fig. 2-10). I observed similar patterns when I considered the thermal exploitation indices. Gravid females tended to have a higher percentage of $T_b > T_{set}$ and a lower percentage of $T_b < T_{set}$ than males or non-gravid females in May and June, but the differences were non-existent or much less pronounced later in the season (Fig. 2-11). Therefore, although the reproductive group by month interactions were non-significant in the statistical analyses because of their complexity and inconsistency, it does seem that the thermoregulatory behaviour of gravid females differed more from that of the other two groups early in the season (in May and June) when gravid females were developing or carrying eggs.

Discussion

Thermal preference

In the thermal gradient, the upper bound of the preferred body temperature range of black rat snakes (29.8°C) was identical to the upper bound for water snakes from the same location (29.8°C), and the lower bound (26.5°C) and mean T_b (28.1°C) for rat snakes were only slightly higher than the lower bound (24.7°C) and mean T_b (27.1°C) of water snakes (Brown and Weatherhead 2000). Lillywhite (1987) reviewed the literature on the preferred body temperatures of snakes and summarised the data available for 55 species from five families. Although a number of different methods were used, for

the majority of the species mean preferred body temperatures were between 28 and 34°C and were often close to 30°C (Lillywhite 1987). Thus, black rat snakes seem to have a mean preferred body temperature at the lower end of the range reported for other snake species. It is possible that species or populations from high latitudes or high altitudes that experience cooler temperatures have lower preferred body temperature ranges than species that experience more favourable climatic conditions.

General patterns of thermoregulation

Black rat snakes in Ontario rarely exploited their thermal environment to its full extent, at least during the day. Black rat snakes thermoregulated carefully at night (or they simply chose the best thermal habitats when they had no conflicting demands) and were always as warm as their environment allowed from 2300h to 600h. However, when averaged for the whole season, their T_bs did not attain the lower bound of T_{set} during the day although the T_es indicated that they could have done so for approximately 10 hrs per day. Even during the warmest month (July), black rat snakes only maintained their T_bs within T_{set} for approximately 7 hrs per day whereas the T_es indicated that T_bs within T_{set} could have been achieved for 14 hrs per day. In July, the snakes' slow heating phase lagged behind the sharp increase in T_es that occurred shortly after sunrise by approximately 3.5 hrs. T_bs also dropped below the lower bound of T_{set} approximately 3 hrs before T_es did. This clearly

indicates that for black rat snakes the costs associated with maintaining T_bs within the boundaries of T_{set} as long as possible during the day outweighed the benefits of doing so. Brown and Weatherhead (2000) also reported a 2-3 h lag time before the morning increase in T_bs in water snakes, but, contrary to rat snakes, water snakes remained within their preferred range as long as possible in the evening. In fact, water snakes were warmer than the warmest model for most of the evening and night, indicating that they were using some thermal habitats that were not sampled by Brown and Weatherhead (2000). One demand that likely conflicted with thermoregulation in rat snakes was foraging, because time spent thermoregulating cannot be spent hunting. Black rat snakes feed mostly on rodents (Fitch 1963, Stickel et al. 1980) that they actively pursue and the most profitable rodents to prey on in my study area are chipmunks (*Tamias striatus*) which are abundant in forests (G. Blouin-Demers, unpublished data), the coolest available habitat. In addition, chipmunks are most active at dawn and dusk (Bider 1968). If black rat snakes are hunting chipmunks in early morning and late evening, they would not be able to spend a lot of time thermoregulating at those times and this could explain the delayed heating and early cooling I observed in rat snakes.

Effectiveness of thermoregulation (*E*)

I am aware of only one previous study (Brown and Weatherhead 2000) that has applied the quantitative indices of thermoregulation developed by Hertz et al. (1993) to snakes. Brown and Weatherhead (2000) were also the first

to use these indices to compare the thermoregulatory behaviour of different segments of a population (i.e., males, non-gravid females, and gravid females). Therefore, I can only compare my results from black rat snakes to the results Brown and Weatherhead (2000) obtained for the northern water snakes (*Nerodia sipedon sipedon*) studied within the same study area. While it is not possible to evaluate the extent to which my results are typical of other snakes, together with Brown and Weatherhead's data for northern water snakes, my results for black rat snakes can serve as benchmarks for future studies of snake thermoregulation.

The calculated index of the effectiveness of thermoregulation (E) for black rat snakes was 0.41, very close to the value of 0.48 reported for water snakes by Brown and Weatherhead (2000). Given the similarity of their estimated Es, and the ranges of potential values of E, both species might be considered moderately precise thermoregulators. However, as recognised by Hertz et al. (1993), a given value of E can result from a variety of d_b and d_e combinations because a ratio enters in the calculation of E (i.e., d_b / d_e). Two species can therefore face different thermal environments and exhibit different thermoregulatory strategies, and still have the same value of E if their ratios between d_b and d_e are the same. Thus, it is also important to consider the magnitude of d_b and d_e in interpreting E. For black rat snakes, d_b was 4.8°C and d_e was 8.1°C, whereas for water snakes d_b and d_e were only 2.4°C and 4.0°C, respectively (Brown and Weatherhead 2000). Thus, water

snake T_bs were, on average, approximately two times closer to their T_{set} than were the T_bs of black rat snakes. Also, the thermal environment faced by water snakes was only about half as challenging from a thermoregulation perspective as the thermal environment faced by black rat snakes. Although the general climatic conditions and weather patterns experienced by the two species are the same, black rat snakes are terrestrial and inhabit mostly forested habitats that receive very little direct solar radiation and those habitats are thus much cooler than the open aquatic habitats inhabited by water snakes. For the three years of the study, the T_es indicated that black rat snakes could have achieved T_bs within T_{set} 44% of the time whereas water snakes could have done so 53% of the time (Brown and Weatherhead 2000). Therefore, black rat snakes and northern water snakes in eastern Ontario have similar indices of thermoregulation effectiveness (E), but they actually face quite different thermal habitats and experience different T_bs. Evidence that thermoregulation costs are substantially higher for rat snakes than for water snakes in Ontario is also provided by the mean T_bs, 25% quartiles, and 75% quartiles maintained by the snakes in the field and in the laboratory. Water snakes had almost identical mean T_bs, 25% quartiles, and 75% quartiles in the thermal gradient and in the field when environmental conditions allowed T_bs within T_{set} to be reached, whereas rat snakes maintained much lower mean T_bs, 25% quartiles, and 75% quartiles in the field than in the thermal gradient.

The effectiveness of thermoregulation index (E) has also been calculated for several lizard species (Table 2-7). Hertz et al. (1993) calculated E for six populations of three *Anolis* species and obtained mean species values ranging from 0.11 to 0.53. Once again, however, we need to consider the magnitude of d_b and d_e to get a meaningful comparison between these lizard species and black rat snakes. Mean d_b varied from 1.1°C to 2.5°C whereas mean d_e varied from 2.3°C to 4.1°C for the three *Anolis* species. These deviations are much smaller than the deviations I calculated for black rat snakes. Similarly, the mean E calculated for four *Varanus* species studied by Christian and Weavers (1996) in Australia was ≈ 0.5, but the mean d_b was only ≈ 2.8°C and the mean d_e ≈ 6.3°C. Thus, although black rat snakes have an overall E similar to the other species that have been studied to date, their T_bs were much further from their T_{set} and their environment was much more challenging from a thermoregulation perspective than for the other species.

Thermal exploitation (Ex)

The thermal exploitation index (Ex) of black rat snakes (22.4%) was only about half the value of Ex of the water snakes (44.4%) studied by Brown and Weatherhead (2000). The Ex of rat snakes is also lower than the Ex that has been calculated for lizards (Table 2-7). Reported Ex values ranged from 77 to 96%, depending on season, for frillneck lizards (*Chlamydosaurus kingii*) studied in Australia (Christian and Bedford 1995). Ex varied from 0 to 100%,

depending on species and season, and averaged ≈ 46% for the four species of monitor lizards (*Varanus*) studied by Christian and Weavers (1996). Thus, black rat snakes do not seem to be exploiting their thermal environment nearly as much as the single other snake species and the few lizard species for which *Ex* values are available.

Group differences in thermoregulation

In viviparous snakes, it has been shown that gravid females thermoregulate more carefully than non-gravid females and males (Charland and Gregory 1990). This difference in thermoregulatory behaviour has also been suggested (but not demonstrated) to cause differences in habitat use, with gravid females preferring habitats that provide the best opportunities for behavioural thermoregulation (Reinert 1993). In Chapter One I showed that gravid female black rat snakes have a stronger preference for edge habitats than non-gravid females and males. My findings in this oviparous species seem to parallel the patterns that have been documented for viviparous species and I thus expected to find that gravid female black rat snakes thermoregulated more carefully than non-gravid females and males. If edges are indeed used for thermoregulation, I predict that males and non-gravid females should thermoregulate better while in edges than while in other habitats.

The lack of significant differences in T_{set} between the three reproductive groups in the laboratory thermal gradient indicated that females do not alter their preferred T_b when gravid. However, several lines of evidence suggested that gravid female rat snakes in the field thermoregulated more carefully than other snake groups. (1) Gravid females maintained significantly higher T_bs during the day than males and non-gravid females. (2) Gravid females had significantly higher *E*s during the day than males and non-gravid females. (3) Gravid females had significantly more T_bs that fell above T_{set} and significantly fewer T_bs that fell below T_{set} than non-gravid females at times when T_{set} could be reached in at least one habitat during the day. To my knowledge, this is the first demonstration that gravid females of oviparous snake species thermoregulate more carefully than non-gravid females and males.

Because gravid female black rat snakes only carry their eggs for a relatively short time (nesting occurs during July), I had anticipated that the difference in thermoregulatory behaviour between reproductive groups should be apparent only in the early part of the season when females are actually carrying eggs. I had also anticipated that this difference should be less pronounced than in viviparous species where gravid females carry their offspring for most of the active season because in oviparous species a large proportion of embryo development occurs while the eggs incubate in nesting sites. One counter-intuitive and recurrent result was the lack of significant

interactions between reproductive groups and months in my statistical analyses of the effect of these factors on thermoregulatory behaviour. The interaction patterns between reproductive groups and months were complex and inconsistent, and therefore non-significant in the analyses. However, I did find evidence that the differences in thermoregulatory behaviour between gravid females and the other snake groups were much more pronounced during May and June when female rat snakes are actually carrying their eggs. In the northern water snakes studied by Brown and Weatherhead (2000), the differences in thermoregulation between reproductive females and the other snake groups were most obvious during July and August and usually non-existent during the early part of the season. Thus, reproduction affects thermoregulation of females in both viviparous and oviparous snake species, and the timing of the change in thermoregulatory behaviour coincides with the time that the advantage of maintaining high T_bs is greatest.

Problems with the current indices of thermoregulation

The ratio nature of *E* presents other problems in addition to the one discussed above, where several species that experience different climatic conditions and have different thermoregulatory behaviour have very similar *E*s. In a statistical sense, ratios are rarely desirable because they are sensitive to extreme values in the numerator and the denominator (a problem noted by Christian and Weavers 1996) and their sampling distribution is severely

skewed, which creates serious statistical artefacts. For example, if two thermoconformers both have d_bs only 0.1°C lower than the *de* of their respective habitat, and one occupies an area that is thermally very challenging (d_e = 10) and the other an area that is very benign (d_e = 0.1), their respective E will be almost pure thermoconformer (E = 1- 9.9/10 = 0.01) and pure thermoregulator (E = 1 - 0/0.1 = 1). Thus, this index performs poorly in describing the thermal ecology of species that live in benign habitats such as the tropics, and this accounts for the majority of reptile species (Shine and Madsen 1996), because even species that are close to being thermoconformers ($d_b \approx d_e$) get moderate to high Es when d_e is small. In addition, this index performs poorly at describing the thermal ecology of thermoregulators ($d_b << d_e$) inhabiting challenging habitats because E remains low to moderate when d_e is large. In summary, there are several problems associated with E: (1) it is not defined if the thermal environment is perfect (d_e = 0), (2) it is impossible to interpret without considering the respective magnitude of d_b and d_e, and (3) it can give spurious representations of the thermal ecology of a species because it uses a ratio (see sample calculation above). For these reasons, I recommend abandoning the use of E in the study of thermoregulation. I propose a simpler index of the effectiveness of thermoregulation defined as the difference between d_e and d_b. This index provides an open-ended scale where negative numbers represent animals that avoid thermally favourable habitats, 0 represents perfect thermoconformity, and positive numbers

represent animals that thermoregulate to some extent. The magnitude of the difference is a measure of how much an animal departs from thermoconformity and thus is an index of the effectiveness of thermoregulation.

Christian and Weavers (1996) also proposed alternatives to *E*. Their thermal exploitation index (*Ex*) is such an alternative. *Ex* quantifies the extent to which a species exploits its thermal opportunities. In addition to using *Ex*, Christian and Weavers (1996) also calculated *E* only for the time periods where T_bs within the T_{set} range are available and judged that, under these conditions, *E* provided an acceptable index of the effectiveness of thermoregulation. However, these two alternatives on their own are also incomplete because they give no indication on how thermally challenging the environment is or how closely T_bs match T_{set} when T_es within T_{set} are not available. Thus, to get a complete picture of the thermal ecology of a species, I propose that (1) d_e should be used to quantify the thermal quality of the habitat, (2) d_b should be used to determine the accuracy of T_b, (3) $d_e - d_b$ should be used to quantify how much departure there is from perfect thermoconformity (the effectiveness of thermoregulation), (4) and the modified *Ex* (Brown and Weatherhead 2000) should be used to determine the extent to which a species exploits the available opportunities for behavioural thermoregulation.

Cost-benefit thermoregulation model

The cost-benefit model of thermoregulation predicts that ectotherms living in habitats where it would be too costly to thermoregulate precisely should be thermoconformers (Huey and Slatkin 1976). Because my study population of black rat snakes is at the northern extreme of the species' distribution and is also close to the northern limit of reptile distributions, I had predicted that their thermoregulation costs should be very high and thus they should be thermoconformers. The environment inhabited by black rat snakes was indeed much more challenging from a thermoregulation perspective than the environment inhabited by other reptiles studied so far (see above). However, the calculations of the effectiveness of thermoregulation (E) indicated that black rat snakes were moderate thermoregulators and fell within the same range as other species that inhabit more benign climates. Conversely, the thermal exploitation index (Ex) calculated for black rat snakes in Ontario was only about half the Ex for other species that inhabit less thermally challenging habitats, indicating that black rat snakes exploited their thermal environment less than these other species. One problem in defining the thermoregulatory strategy of an ectotherm is that a strategy cannot be defined by a single parameter, but actually requires a set of different parameters (Hertz et al. 1993). Thus, whether or not my results for black rat snakes support the current cost-benefit model of thermoregulation depends on which parameters I use to define their

thermoregulatory strategy. Based on their effectiveness of thermoregulation (E), rat snakes should be classified as moderately precise thermoregulators despite their very challenging thermal environment and hence the current cost-benefit model would not be supported. Conversely, based on their thermal exploitation (Ex), they are closer to being thermoconformers and thus would lend more support to the current model.

Shine and Madsen (1996) have already shown that thermoconformity can occur in species that live in tropical areas, where T_e in the range of T_{set} are always available at a low thermoregulatory cost. Snakes in those areas are thermoconformers although they experience very small variation in T_b (stenothermy). My results suggest this seems to be a generalised phenomenon and that as the environment becomes less challenging, ectotherms invest less in thermoregulation. Thus, it appears that all ectotherms try to attain their T_{set} to some extent, but this requires very little effort when the thermal environment is benign (in the tropics) and a lot of effort when the thermal environment is challenging (at high latitudes or high altitudes). One potential explanation for this pattern is that the physiological costs of thermoconformity are very low when the thermal environment is benign because, even without thermoregulation, T_bs are close to T_{set}. The current cost-benefit model of thermoregulation (Huey and Slatkin 1976) considered that the cost of thermoregulation increases as the thermal quality of the habitats decreases, but never considered that the cost of thermoconformity

also increases as the thermal quality of the habitat decreases. The available data on reptilian thermoregulation suggest that the cost of thermoconformity might be more important than the cost of thermoregulation.

The factor that might vary with the quality of the thermal environment is the extent to which species experience T_bs within their T_{set} (quantified by Ex). Presumably, as the thermal environment becomes more challenging and the costs of thermoregulation rise, ectotherms cannot afford to spend as much time within their preferred range. More studies of ectotherms in climatic extremes (both tropical and cool-temperate) employing standard indices of thermoregulation are needed before we can better assess the robustness of this result, but the available data suggest that the current cost-benefit model of thermoregulation is not broadly applicable.

Table 2-1: Mean T_b selected, 75% quartile, and 25% quartile in °C for males, non-gravid females, and gravid females in the laboratory thermal gradient. Means are presented ±1 SE and the range is given in parentheses.

Group	N	Mean T_b	75% quartile	25% quartile
Males	9	27.4 ±0.94	29.6 ±0.99	25.5 ±1.05
		(25.0 – 30.7)	(27.6 – 32.2)	(22.3 – 29.5)
Non-gravid females	21	28.3 ±0.62	29.9 ±0.65	26.8 ±0.69
		(22.5 – 31.7)	(23.5 – 33.4)	(21.5 – 31.0)
Gravid females	11	28.2 ±0.85	30.0 ±0.90	26.6 ±0.95
		(19.5 – 32.8)	(20.9 – 33.7)	(18.0 – 32.3)

Table 2-2: Multiple regression equations used to predict model snake temperature in the different habitats available to black rat snakes and their statistical significance. The variables entered as predictors in the models were air temperature in °C (T), solar radiation in kW/m^2 (R), wind speed in m/s (W), and rainfall in mm/h (RA).

Habitat	Equation	R^2	Significance
Forest	1.16T + 0.66R - 0.02W - 4.07	0.86	$F_{(3,764)} = 1567.15$ $p = 0.0001$
Field/Wetland	1.25T + 31.12R - 9.44	0.94	$F_{(2,587)} = 4337.20$ $p = 0.0001$
Rock outcrop	1.42T + 24.39R + 0.18W -7.60	0.89	$F_{(3,717)} = 1882.68$ $p = 0.0001$
Bare ground in open	1.26T + 28.63R - 0.08W -5.83	0.94	$F_{(3,1002)} = 5083.90$ $p = 0.0001$
Barn	0.77T -0.50R + 0.48RA + 6.19	0.56	$F_{(3,817)} = 347.92$ $p = 0.0001$
Under flat rock	1.06T -11.36R + 0.06W + 3.68	0.92	$F_{(3,284)} = 1126.70$ $p = 0.0001$

Table 2-3: Mean ±1 SE operative temperatures recorded in each habitat available to black rat snakes. Mean ±1 SE deviations of operative temperatures from the preferred body temperature range of black rat snakes in each habitat.

Habitat	mean T_e	mean d_e
Forest	17.5 ±0.06	9.2 ±0.06
Field/Wetland	20.8 ±0.13	12.0 ±0.07
Rock outcrop	25.9 ±0.13	9.5 ±0.07
Bare ground in open	23.3 ±0.12	10.3 ±0.06
Barn	20.4 ±0.04	6.2 ±0.04
Under flat rock	21.5 ±0.05	5.5 ±0.04
Edge forest-field/wetland	-	7.5 ±0.06
Edge forest-rock outcrop	-	5.6 ±0.06
Edge forest- water body	-	7.0 ±0.06

Table 2-4: Summary results of the two-way ANCOVA's for the effects of mean monthly operative temperature, month, and reproductive group on mean body temperatures of black rat snakes for the whole day, daytime only, and night only.

Period	Predictor	Significance
Day	Mean T_e	$F_{(1,321)} = 7.101$ $p = 0.008$
	Month*group	$F_{(8,321)} = 0.420$ $p = 0.909$
	Month	$F_{(4,321)} = 6.774$ $p < 0.001$
	Group	$F_{(2,321)} = 3.275$ $p = 0.039$
Night	Mean T_e	$F_{(1,173)} = 6.888$ $p = 0.010$
	Month*group	$F_{(8,\ 173)} = 0.971$ $p = 0.460$
	Month	$F_{(4,\ 173)} = 7.720$ $p < 0.001$
	Group	$F_{(2,\ 173)} = 1.753$ $p = 0.176$

Table 2-5: Summary results of the two-way ANOVA's for the effects of month and reproductive group on the index of the effectiveness of thermoregulation (E) of black rat snakes for the whole day, daytime only, and night only.

Period	Predictor	Significance
Day	Month*group	$F_{(8,322)} = 0.733$ $p = 0.663$
	Month	$F_{(4,322)} = 12.054$ $p < 0.001$
	Group	$F_{(2,322)} = 3.543$ $p = 0.030$
Night	Month*group	$F_{(8,322)} = 0.471$ $p = 0.877$
	Month	$F_{(4,322)} = 4.756$ $p = 0.001$
	Group	$F_{(2,322)} = 1.004$ $p = 0.369$

Table 2-6: Summary results of the two-way ANOVA's for the effects of month and reproductive group on the indices of thermal exploitation of black rat snakes for the whole day, daytime only, and night only.

Index	Period	Predictor	Significance
$T_b = T_{set}$	Day	Month*group	$F_{(8,314)} = 1.584\ p = 0.129$
		Month	$F_{(4,314)} = 23.704\ p < 0.001$
		Group	$F_{(2,314)} = 1.703\ p = 0.184$
	Night	Month*group	$F_{(8,119)} = 0.892\ p = 0.525$
		Month	$F_{(4,119)} = 7.667\ p < 0.001$
		Group	$F_{(2,119)} = 0.361\ p = 0.698$
$T_b > T_{set}$	Day	Month*group	$F_{(8,314)} = 1.624\ p = 0.117$
		Month	$F_{(4,314)} = 32.610\ p < 0.001$
		Group	$F_{(2,314)} = 3.044\ p = 0.049$
	Night	Month*group	$F_{(8,119)} = 0.367\ p = 0.936$
		Month	$F_{(4,119)} = 8.177\ p < 0.001$
		Group	$F_{(2,119)} = 0.411\ p = 0.664$
$T_b < T_{set}$	Day	Month*group	$F_{(8,314)} = 0.697\ p = 0.694$
		Month	$F_{(4,314)} = 51.023\ p < 0.001$
		Group	$F_{(2,314)} = 4.413\ p = 0.013$
	Night	Month*group	$F_{(8,119)} = 0.570\ p = 0.801$
		Month	$F_{(4,119)} = 10.952\ p < 0.001$
		Group	$F_{(2,119)} = 0.680\ p = 0.509$

Table 2-7: Summary of the available information on the thermoregulatory behaviour of several populations and species under different environmental conditions from studies that have used quantitative indices of thermoregulation.

Species	Habitat	Elevation	Period	d_b	d_e	E	Ex
Anolis cooki	Desert	5 m	Winter	0.8	2.4	0.67	
			Summer	1.3	2.1	0.38	
Anolis cristatellus	Desert	5 m	Winter	2.0	2.3	0.13	
			Summer	0.9	2.5	0.64	
	Mesic	90 m	Winter	3.4	4.6	0.26	
			Summer	1.1	1.2	0.08	
		1150 m	Winter	5.0	9.2	0.46	
			Summer	2.5	5.0	0.50	
Anolis gundlachi	Mesic	210 m	Winter	0.9	0.9	0.00	
			Summer	0.6	0.7	0.14	
		1130 m	Winter	4.9	5.3	0.08	
			Summer	2.2	2.8	0.21	
Varanus panoptes	Tropical	?	Summer	2.0	7.0	0.71	12
			Winter	2.0	6.0	0.67	68
Varanus gouldi	Tropical	?	Summer	1.0	6.5	0.85	96
			Winter	6.0	5.5	-0.09	0

Table 2-7: Continued

Varanus mertensi	Tropical	?	Summer	1.0	9.0	0.89	72
			Winter	3.5	4.0	0.13	74
Varanus rosenbergi	Temperate	?	Summer	4.0	6.0	0.33	95
Nerodia Sipedon	Temperate marsh	200 m	Summer	2.4	4.0	0.42	44
Elaphe obsoleta	Temperate forest	200 m	Summer	4.8	8.1	0.41	22

Note: Data for *Anolis* are from Hertz et al. (1993), data for *Varanus* are from Christian and Weavers (1996), data for *Nerodia* are from Brown and Weatherhead (2000), and data for *Elaphe* are from the present study.

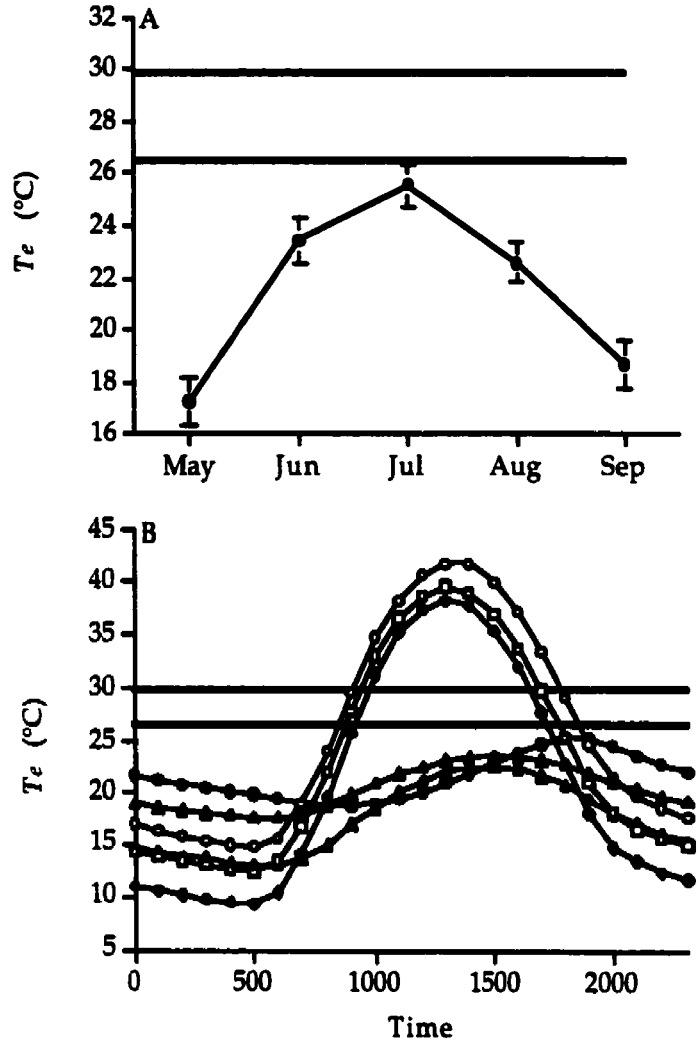

Fig. 2-1: Mean operative environmental temperatures ($T_e \pm 1$ SE) for each month of the active season (A) and mean operative environmental temperatures for each hour of the day under flat rocks (filled circles), inside barns (filled triangles), on rock outcrops (open circles), in forests (open triangles), on bare ground in the open (open squares), and in fields (open lozenges) in Ontario (B). The range of preferred body temperatures (T_{set}) for black rat snakes in Ontario is represented by the horizontal solid lines.

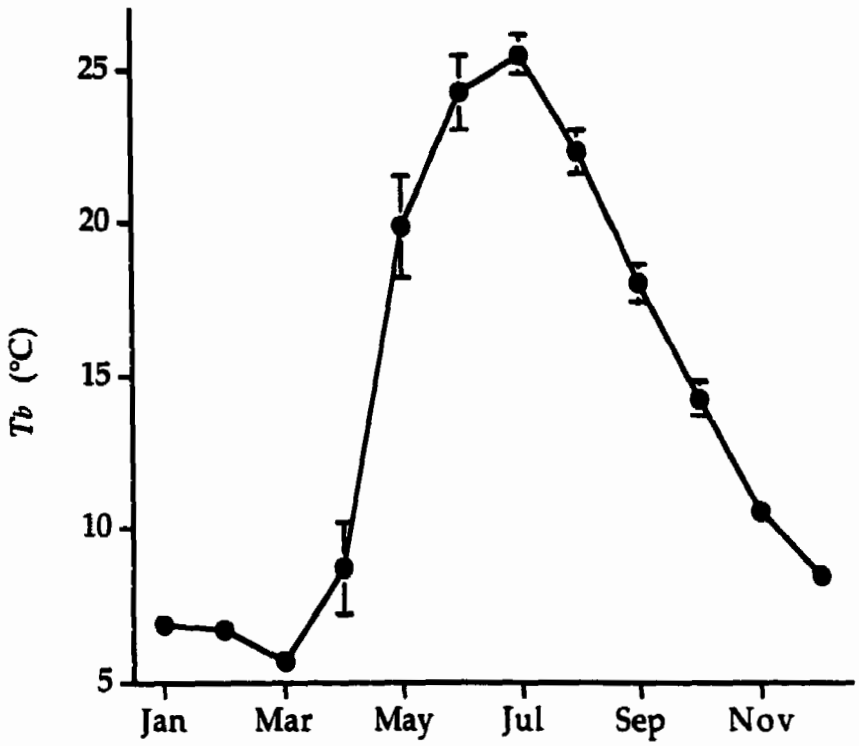

Fig. 2-2: Mean body temperatures ($T_b \pm 1$ SE) maintained by black rat snakes in Ontario for each month of the year.

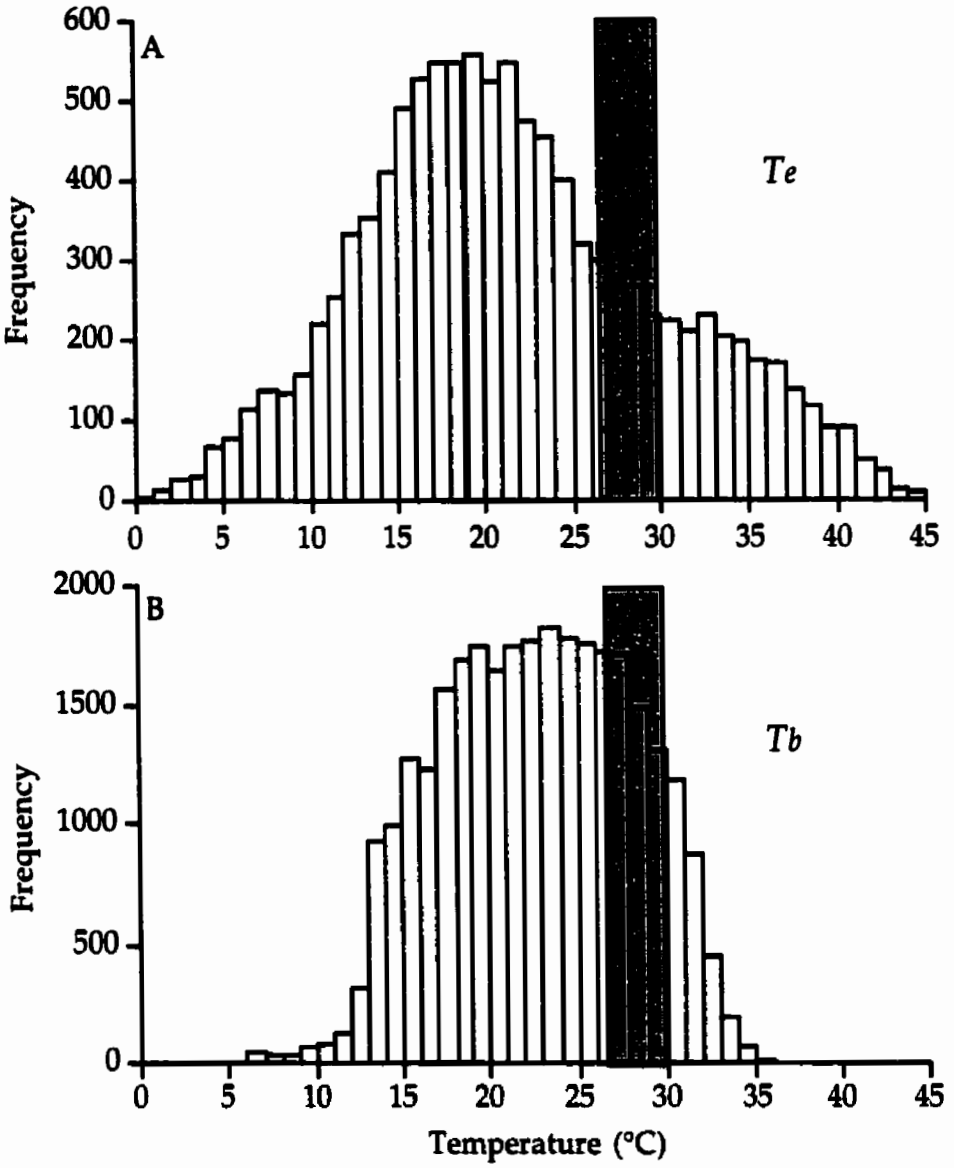

Fig. 2-3: Frequency distribution of hourly mean operative environmental temperatures (T_es) (A) and hourly mean body temperatures (T_bs) (B) of black rat snakes in Ontario. The shaded area represents the range of preferred body temperatures (T_{set}) of black rat snakes in Ontario.

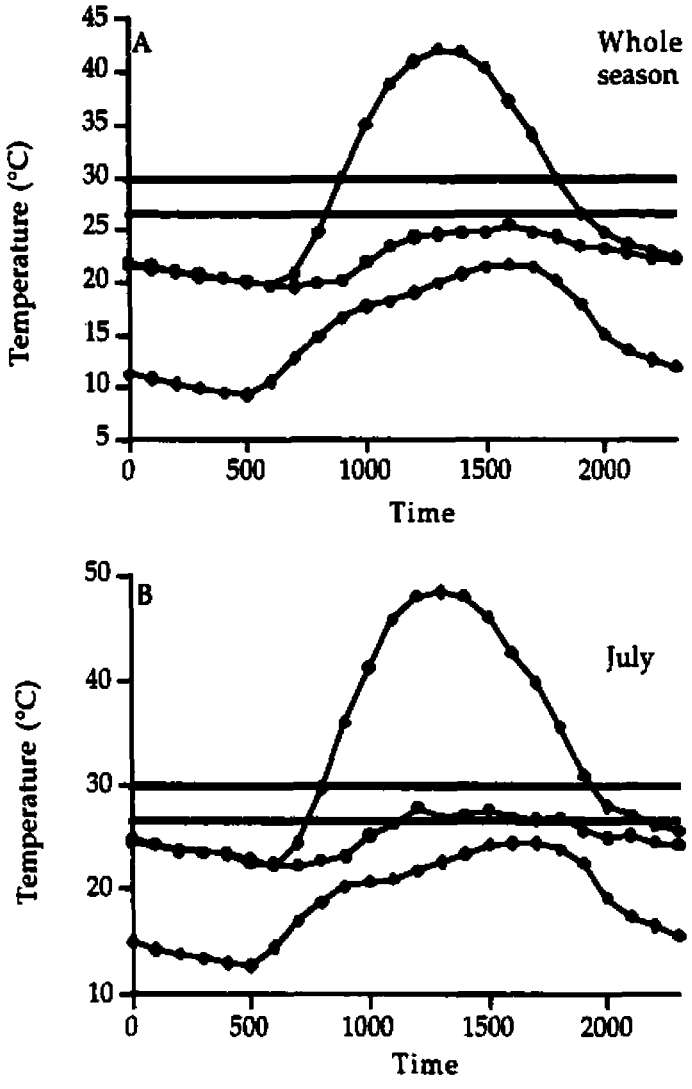

Fig. 2-4: Hourly mean body temperatures (T_bs) (circles) of black rat snakes in Ontario and hourly mean maximum and minimum operative environmental temperatures (T_es) (lozenges) for the whole season (A) and for July only (B). The range of preferred body temperatures (T_{set}) for black rat snakes in Ontario is represented by the horizontal solid lines.

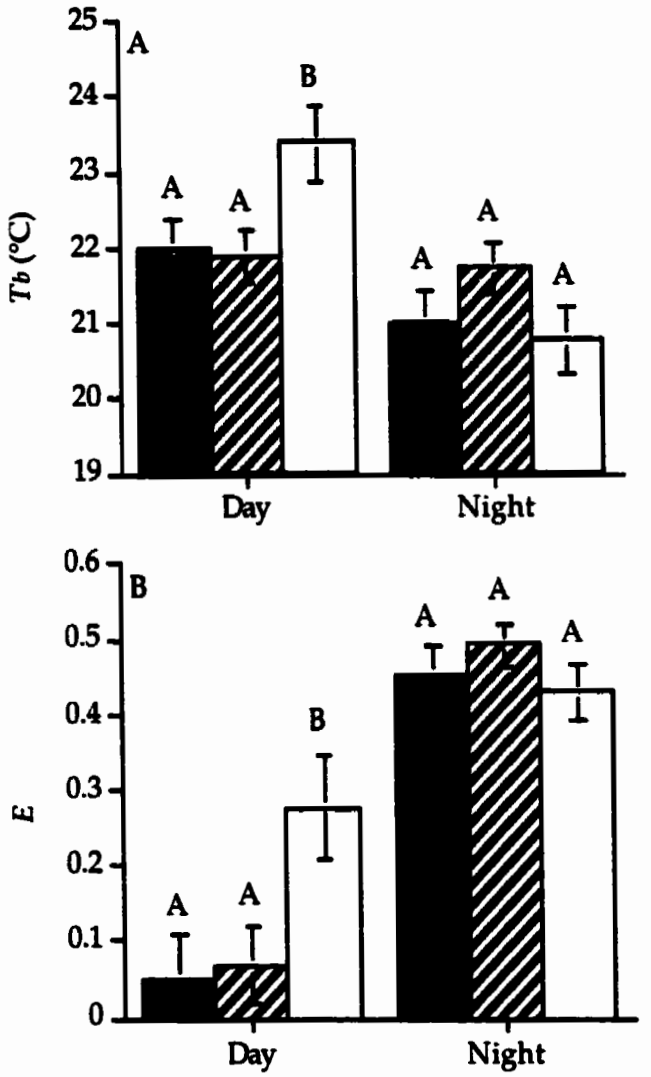

Fig. 2-5: Mean body temperatures (T_bs ± 1 SE) (A) and mean index of thermoregulation effectiveness (E ± 1 SE) (B) for the day and night maintained by male (filled bars), non-gravid female (hatched bars), and gravid female (open bars) black rat snakes in Ontario. Means with the same letters are deemed not significantly different based on Tukey-Kramer HSD tests.

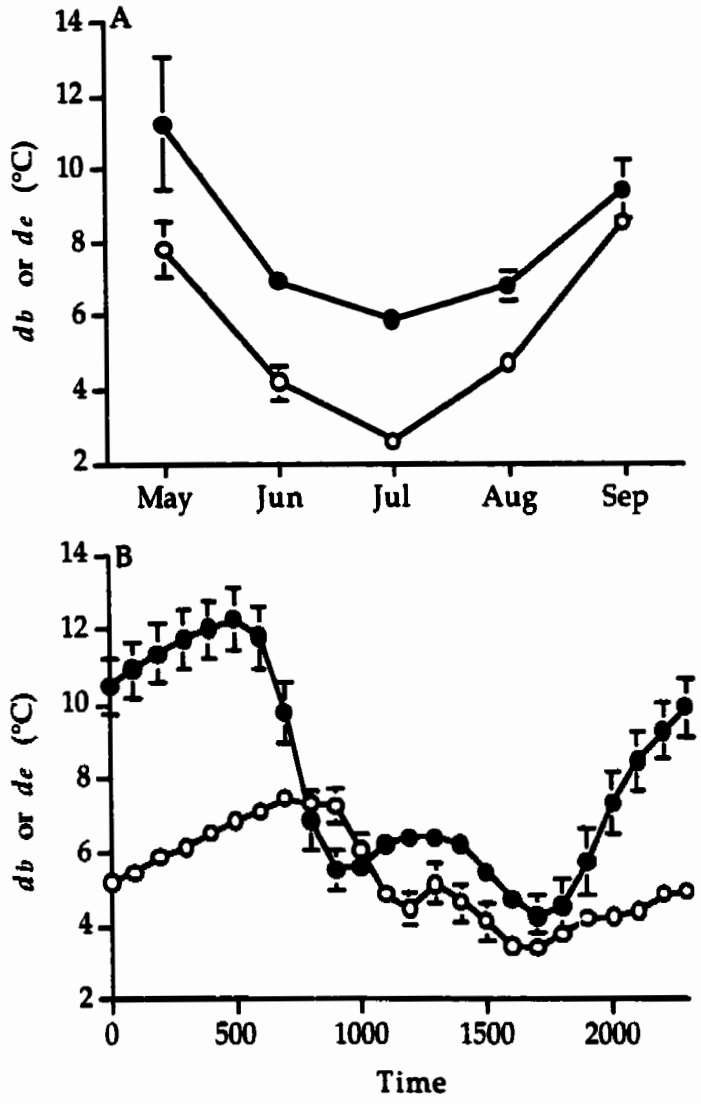

Fig. 2-6: Mean deviations of body temperatures from the range of preferred body temperatures ($d_b \pm 1$ SE) (open circles) of black rat snakes in Ontario and mean deviations of operative environmental temperatures from the range of preferred body temperatures ($d_e \pm 1$ SE) (filled circles) for each month of the active season (A) and for each hour of the day (B).

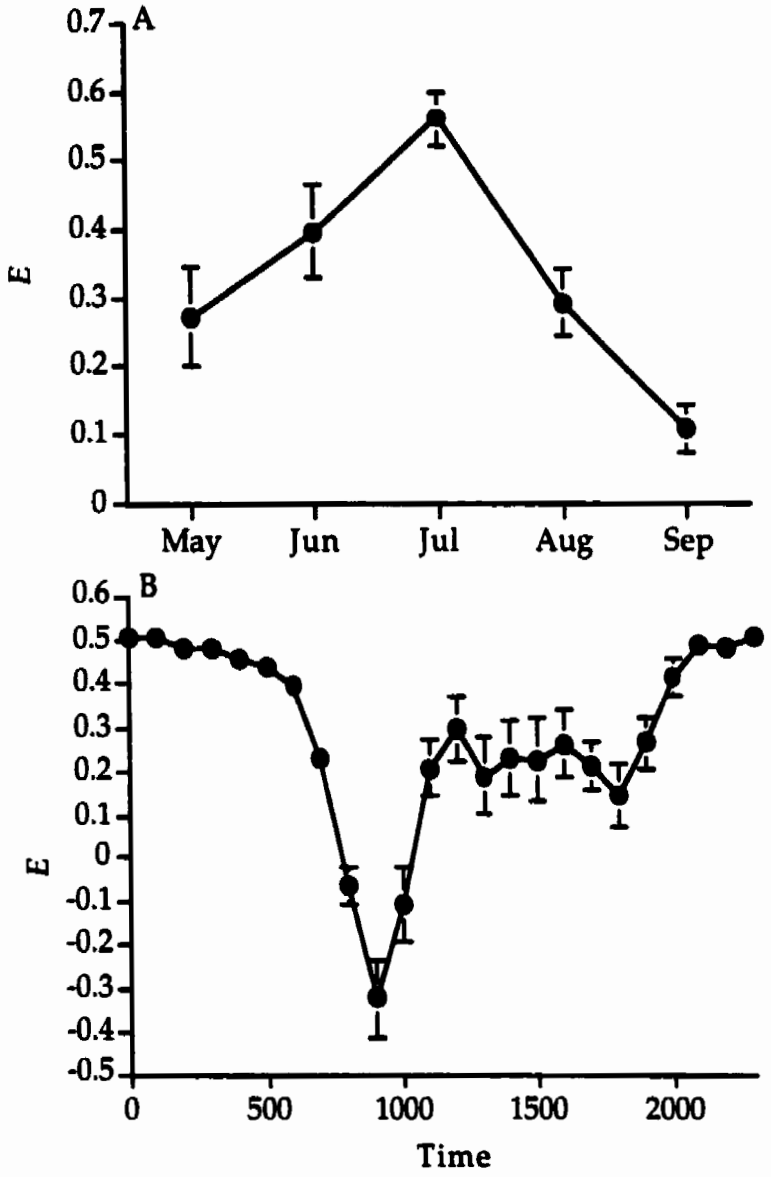

Fig. 2-7: Mean index of thermoregulation effectiveness ($E \pm 1$ SE) of black rat snakes in Ontario for each month of the active season (A) and for each hour of the day (B).

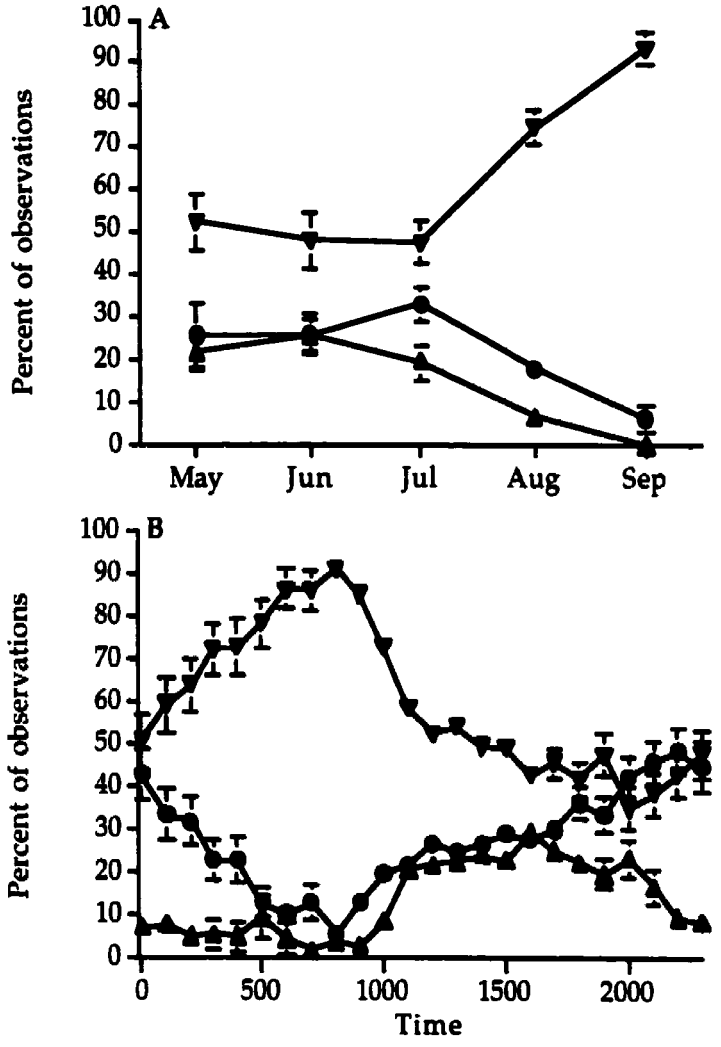

Fig. 2-8: Mean percentage of body temperatures (T_bs) that fell within the range of preferred body temperatures (T_{set}) (circles), above the range of preferred body temperatures (upward triangles), and below the range of preferred body temperatures (downward triangles) for black rat snakes in Ontario when environmental conditions allowed body temperatures within the range of preferred body temperatures to be reached for each month of the active season (A) and for each hour of the day (B). Error bars indicate ± 1 SE

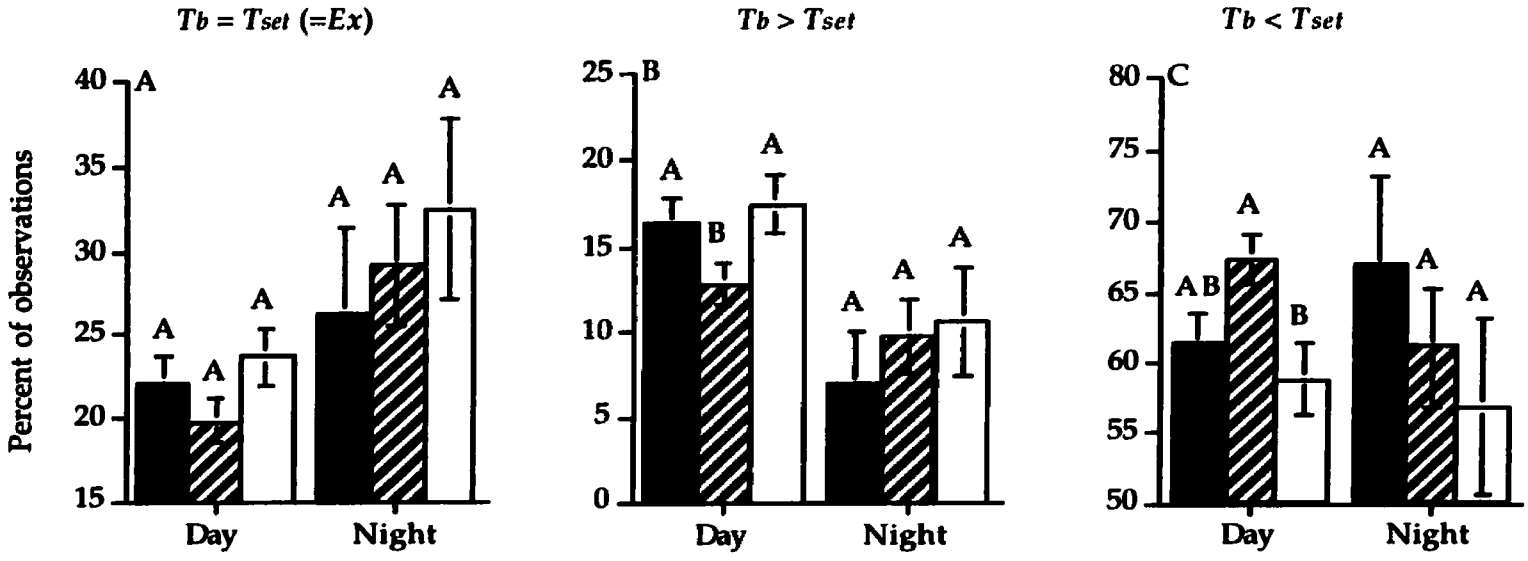

Fig. 2-9: Mean percentage of body temperatures (T_bs ± 1 SE) that fell within the range of preferred body temperatures (T_{set}) (A), above the range of preferred body temperatures (B), and below the range of preferred body temperatures (C) when environmental conditions allowed body temperatures within the range of preferred body temperatures to be reached for the day and night for male (filled bars), non-gravid female (hatched bars), and gravid female (open bars) black rat snakes in Ontario. Means with the same letters are deemed not significantly different based on Tukey-Kramer HSD tests.

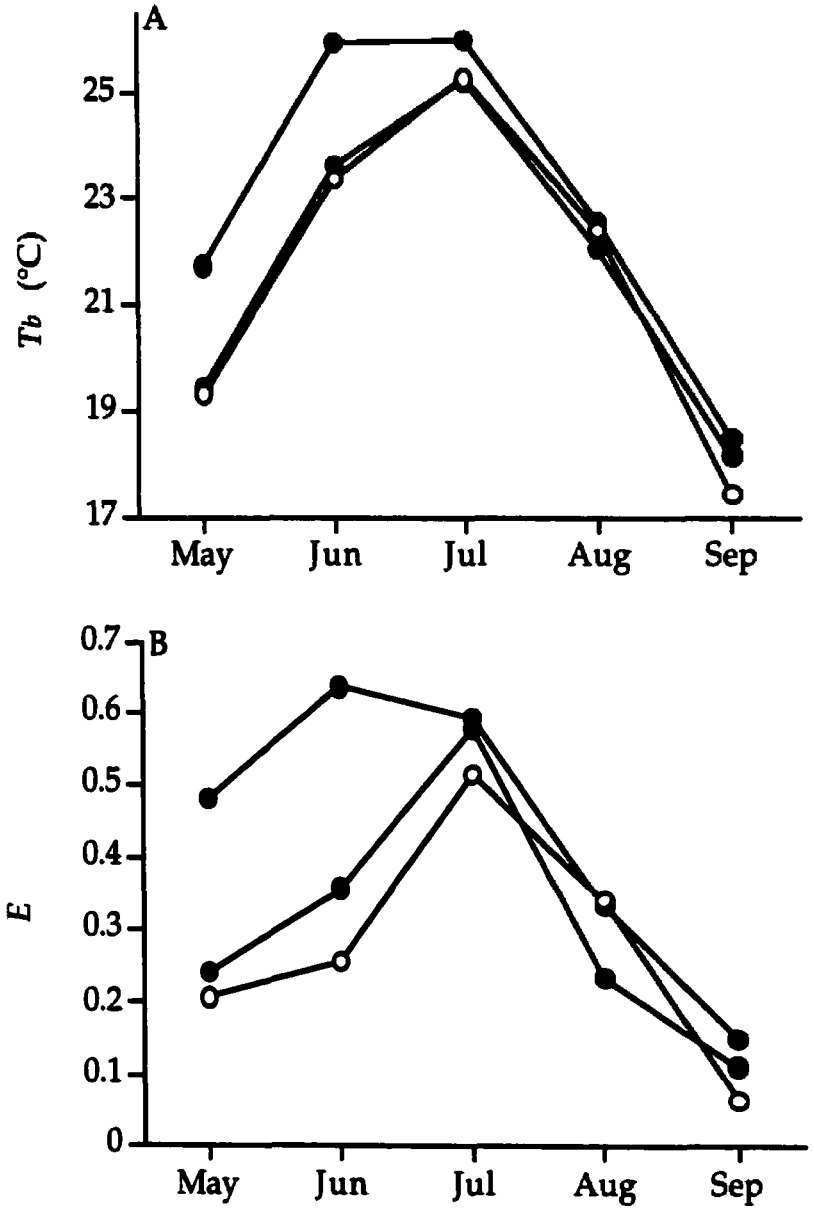

Fig. 2-10: Mean body temperatures (T_bs) (A) and mean index of thermoregulation effectiveness (E) (B) for each month of the active season for male (white), non-gravid female (grey), and gravid female (black) black rat snakes in Ontario.

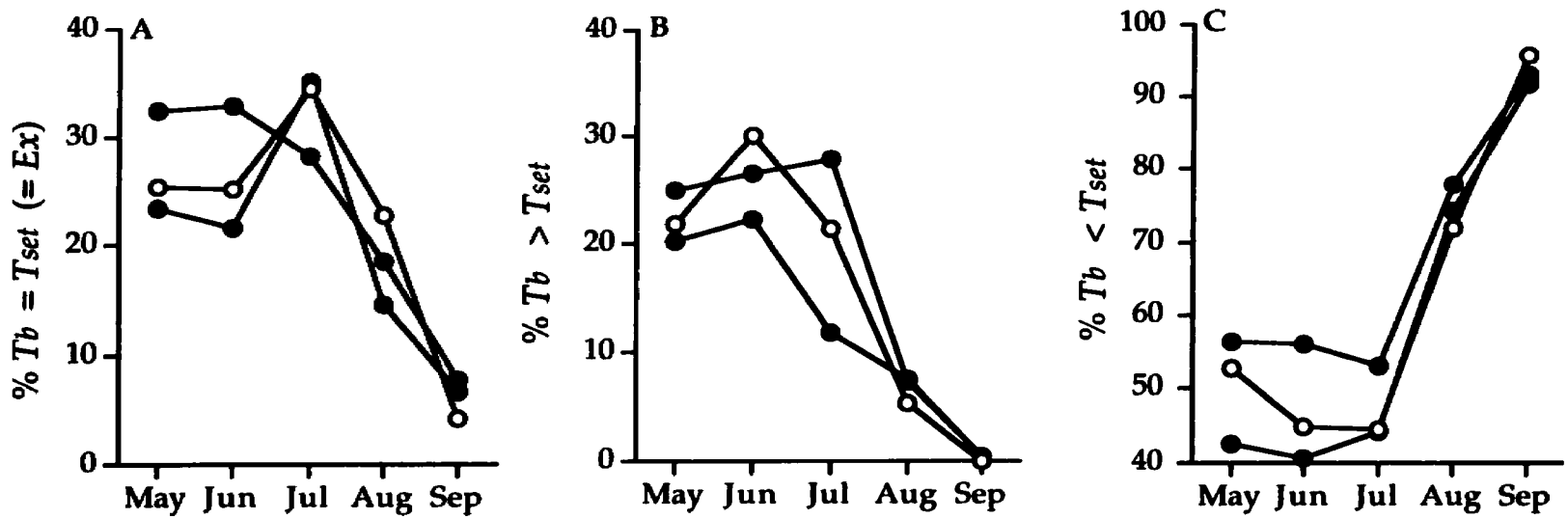

Fig. 2-11: Mean percentage of body temperatures (T_bs) that fell within the range of preferred body temperatures (T_{set}) (A), above the range of preferred body temperatures (B), and below the range of preferred body temperatures (C) when environmental conditions allowed body temperatures within the range of preferred body temperatures to be reached for male (white), non-gravid female (grey), and gravid female (black) black rat snakes in Ontario.

CHAPTER THREE

Thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*) in different habitats

Introduction

Because all physiological processes of ectotherms are temperature dependent, variation in their body temperature (T_b) affects their physiological and behavioural processes (Christian and Tracy 1981, Hertz et al. 1982, Arnold and Bennett 1984). Therefore, virtually all aspects of reptile ecology are affected by T_b variation and T_b ultimately has an impact on fitness (Huey and Kingsolver 1989). In terrestrial ectotherms, T_b regulation is achieved behaviourally by adjusting habitat selection and timing of activity (Huey et al. 1989, Grant 1990). The discovery of behavioural thermoregulation (Cowles and Bogert 1944) has led to the tenet that thermoregulation is the single most important proximate factor influencing habitat selection by terrestrial squamates (Reinert 1993). One expectation arising from this perspective is that squamates should preferentially use thermally superior habitats, particularly when thermal demands are high (e.g., during gestation or digestion). Direct evidence in support of this expectation is limited (Reinert 1984a,b, Shine and Madsen 1996), but results presented in Chapter One and Chapter Four suggest that thermoregulation plays an important role in habitat selection by black rat snakes.

There is also a second, and less obvious issue that arises from the view that snakes vary habitat use, at least in part, to help regulate their T_b. Stated simply, do snakes thermoregulate differently when in different habitats? For

instance, while in the thermally superior habitats snakes may thermoregulate precisely because it is easy to do so, while in thermally inferior habitats they may become thermoconformers because it is too expensive in time or energy to attempt to regulate T_bs behaviourally in those habitats. Alternatively, they may invest equally in thermoregulation in all habitats, and achieve more favourable T_bs in the thermally superior habitat simply because those temperatures are encountered more often. I am unaware of any study that has investigated how ectotherms thermoregulate in habitats varying in thermal quality. My general goal in this chapter is to investigate this issue in black rat snakes (*Elaphe obsoleta obsoleta*).

In Chapter One I demonstrated that black rat snakes prefer habitat edges such as the boundaries between forest and open habitats, including ponds, wetlands, and rock outcrops. These analyses confirmed the results of previous studies that had shown that rat snakes prefer edges in human-modified landscapes (Weatherhead and Charland 1985, Durner and Gates 1993) and extended the results to more pristine landscapes. In Chapter One I also showed that in black rat snakes the preference for edges was more pronounced in gravid females than in non-gravid females and males. I hypothesised that gravid female black rat snakes prefer edges because edges offer better thermoregulatory opportunities. I provided indirect support for this hypothesis by ruling out other potential reasons that gravid females might be attracted to edges: edges are not used primarily for foraging because

female black rat snakes do not feed while gravid, and edges are not used preferentially as retreats from predators because retreat sites used by black rat snakes were as numerous in the forest as in edges. Finally, I proposed that more direct support for my hypothesis that edges are used for thermoregulation would be provided if it was shown that gravid females thermoregulate more carefully than either males or non-gravid females.

In Chapter Two I demonstrated that gravid female black rat snakes did indeed maintain higher T_bs than non-gravid females and males. I also showed that, in relation to the thermal opportunities offered by the environment, gravid females thermoregulated more effectively and exploited the thermal environment more than males and non-gravid females. These data further strengthened my assertion that edges are used for thermoregulation. However, thus far I have not demonstrated empirically that edges are of higher thermal quality than other habitats available to black rat snakes, so my first objective in this chapter was to test that prediction. If edges are preferred because they offer better opportunities for thermoregulation, then snakes should have T_bs closer to their preferred body temperature range (T_{set}) while in edges than while in other habitats, independent of their reproductive status. My second objective in this chapter was to test this prediction.

If snakes maintain T_bs closer to their T_{set} while in edge habitat, they could do so in two ways. Snakes could either invest equally in

thermoregulation in all habitats but experience high T_bs in edges because edges are thermally superior, or they could invest more in thermoregulation while in edges than while in other habitats. The latter prediction is consistent with Huey and Slatkin's (1976) cost-benefit model of thermoregulation, which predicts that ectotherms should invest more in thermoregulation when the costs of thermoregulating are low. The main cost of thermoregulation is time, because time invested in thermoregulation cannot be invested in other activities, such as mate-searching or prey acquisition. Therefore, my final objective was to test the prediction that black rat snakes invest more into thermoregulation while in thermally superior habitats.

Materials and Methods

Study area, study animals, and radio-telemetry

I conducted this study from 1997 to 1999 at the Queen's University Biological Station on the shore of Lake Opinicon, approximately 40 km north of the city of Kingston (Ontario, Canada). The study area was approximately 9.5 km by 2.5 km and was mostly covered by second-growth deciduous forest (Chapter One). Black rat snakes were captured both at communal hibernacula during spring emergence (Blouin-Demers et al. 2000b) and opportunistically during the remainder of the active season (Prior et al. 2001). From all the individuals captured, I selected a subset of individuals for surgical implantation of temperature-sensitive radio-transmitters (Model SI-2T,

Holohil Systems Inc., Carp, Ontario). Implantation was done under isoflurane anaesthesia (Appendix One). Transmitters weighed 8.6 g (maximum ratio of transmitter mass : body mass = 0.025) with a battery life of 20 months at 20 °C. Snakes were released three days following surgery and thereafter were located every second day using a radio-receiver (TRX-2000S, Wildlife Materials Inc., Carbondale, Illinois). All locations of snakes were categorised in general habitat categories (artificial edge, natural edge, field, forest, wetland, and barn) and recorded using a GPS unit (Pathfinder, Trimble Navigation Ltd, Sunnyvale, California) with sub-meter accuracy in the field (Chapter One).

To measure the T_bs of black rat snakes, I took advantage of the temperature-sensing capabilities of the SI-2T radio-transmitters. Calibration curves relating radio-transmitter pulse rate to temperature were supplied by the manufacturer for each transmitter and I verified the accuracy of the curves before using them (Chapter Two). I positioned two automated radio-telemetry data loggers (SRX 400, Lotek Engineering Inc., Newmarket, Ontario) in the study area to record T_bs of black rat snakes every 10 min throughout the day each year. However, because the snakes regularly moved out of range of the data loggers, it was not possible to obtain complete T_b profiles for each individual. From May 1997 to November 1999 I followed 17 males and 36 females for periods ranging from 1 to 30 months. Twenty-three snakes (nine males and 14 females) were followed in multiple years and therefore I have data for 79 "snake years" (25 "male years" and 54 "female years").

Thermal quality of habitats

I constructed physical models of black rat snakes using 40 cm-long pieces of copper pipe painted black to measure the operative environmental temperatures (T_es) available to rat snakes in the different habitats within my study area. I ensured that the models accurately predicted the T_b of black rat snakes using carcasses of rat snakes freshly killed on the road (Chapter Two). I attempted to measure T_es in all the microhabitats available to black rat snakes within all of the different habitats. However, it would be impossible to measure every nuance in the thermal quality of microhabitats within habitats because of the inherent heterogeneity of natural habitats. Also, the thermal heterogeneity is much smaller among microhabitats within a habitat than among habitats (except for edges). Therefore, I deployed the models in at least four of the most common microhabitats in each of the different habitats and left them in place for several weeks. I used multiple regression to build predictive equations of the mean model temperature in all microhabitats within the different habitats based on climatic data collected hourly at the Queen's University Biological Station (Chapter Two). I used these equations to generate mean T_es for each habitat each hour for the duration of the study.

My aim was to measure T_es in all the habitats available to black rat snakes. The different habitats in the study area could be classified as forest, rock outcrop, field, wetland, and water body (Chapter One). I measured T_es by placing the model snakes in forests, rock outcrops and fields. I excluded open

water because rat snakes are terrestrial. I excluded wetlands because in my study area, from a black rat snake's perspective wetlands (mostly dry with thick growth of sedges) are structurally similar to fields. Thus, I considered the T_es for wetlands to be the same as for fields. There were also a number of retreat sites (rock piles, crevices in rock outcrops, large logs, old barns, old machinery, inside snags) that were used by all snakes and that could not be adequately classified in any of the above categories. Therefore, I also placed the portable model in two representative and commonly used retreat sites (under flat rocks on rock outcrops and in barns) to measure the T_es available to black rat snakes in such retreats (Chapter Two).

To quantify the thermal quality of the different habitats, I used the thermal quality index (d_e) introduced by Hertz et al. (1993). These authors defined d_e for a given habitat as the mean of the deviations of T_es from T_{set} in that habitat. If T_e is below the preferred T_b range, d_e is the difference between the lower bound of T_{set} and T_e and if T_e is above the preferred T_b range, d_e is the difference between T_e and the upper bound of T_{set}. In Chapter Two, using data for 41 black rat snakes placed individually in a laboratory thermal gradient, I determined the preferred T_b range (T_{set}) to be from 26.5°C to 29.8°C for the study population. I used these values for T_{set} in the present calculations.

In Chapter One I showed that the preferred habitats of the black rat snakes I studied were the edges between the forest and open habitats (e.g.,

fields, wetlands), where I defined an edge as extending 15 m on each side of the boundary between forest and any open habitat. I evaluated the thermal quality of edges assuming that snakes had access to both sides of the boundary at no cost. Because the displacements involved in shuttling between the sunny open habitat and the forest shade are extremely short in an edge, this seemed like a reasonable assumption. T_es in Ontario forests almost never exceed the upper bound of T_{set} and offered a permanent refuge against high temperatures (Chapter Two). Therefore, in determining the thermal quality of edges, I was only interested in instances where the lower bound of T_{set} could not be reached on either side of the boundary, because overheating was never a concern in the forest. Hence, I defined d_e for edges as the minimum deviation of T_e from the lower bound of T_{set} in the forest or in the open habitat (Chapter Two).

Thermoregulation in different habitats

The first and simplest method I used to investigate whether black rat snakes experienced more favourable T_bs while in edges than while in other habitats was to calculate their mean T_b in the different habitats. More refined methods used to quantify thermoregulation require the determination of the "accuracy" of T_b, or the extent to which an ectotherm experiences T_bs within its T_{set}. Following Hertz et al. (1993), I used d_b as such an index. Similarly to d_e, d_b is defined as the mean of the deviations of T_bs from T_{set}. These data

were used to test the prediction that black rat snakes enjoy more favourable T_bs while in edges than while in other habitats.

Based on d_e and d_b, Hertz et al. (1993) designed an index of thermoregulation effectiveness (E) defined as $E = 1 - (\bar{d}_b / \bar{d}_e)$. In Chapter Two I demonstrated that several problems were associated with this index and recommended its abandonment. Instead, I introduced a better index of the effectiveness of thermoregulation, d_e - d_b, which I use here. This index provides an open-ended scale where negative numbers represent animals that use thermally favourable habitats less than their availability, 0 represents perfect thermoconformity, and positive numbers represent animals that thermoregulate to some extent. The magnitude of the difference is a measure of how much an animal departs from thermoconformity, and thus is an index of the effectiveness of thermoregulation. Finally, to get a complete picture of the thermoregulatory behaviour of rat snakes while in edges and while in other habitats, I followed the recommendations I made in Chapter Two and I also used an index of thermal exploitation (Ex) first introduced by Christian and Weavers (1996). Ex is defined as the percentage of T_bs that fall within T_{set} during time periods where the T_es indicate that the lower bound of T_{set} could have been reached. I calculated the different thermoregulation indices separately for each habitat to compare black rat snake thermoregulation while in these different habitats. I also calculated the indices separately for day (600h - 1800h) and night (1800h - 600h) because black

rat snakes have been shown to thermoregulate differently during those time periods (Chapter Two).

In calculating indices of thermoregulation for black rat snakes, I wanted to determine the effectiveness of thermoregulation (d_e - d_b) in each habitat and the extent to which black rat snakes exploited the thermal opportunities (Ex) offered by each habitat, in relation to the thermal opportunities available in each habitat. Thus, for this analysis I quantified thermoregulation within each habitat regardless of thermal opportunities that might have been available in other habitats and I used T_e or d_e values for that habitat only. For example, while calculating d_e - d_b in forest, I only considered the d_e values of models in the forest. These calculations quantify how much the T_bs of snakes deviate from their T_{set} or how much time they spend with T_bs within T_{set} compared to the T_b deviations or time with T_b within T_{set} they would experience if they used the habitat in which they were randomly (the average deviations of T_es from T_{set} or the average time with T_e within T_{set} in the habitat). Therefore, these calculations determine how selective the snakes were about their microhabitat within a habitat. These data were used to test the prediction that black rat snakes invest more in thermoregulation while in habitats of higher thermal quality.

Because the monitoring interval for T_bs (monitored almost continuously on the data loggers) and the monitoring interval for determining in which habitat the snake was located (monitored at relocation

every 48 hrs) were different, not every T_b measurement recorded could be assigned to a given habitat with certainty. Therefore, I only included in my analyses T_b measurements for snakes that had not changed habitats while the T_bs were recorded. However, I excluded data collected during intervals in which a snake had changed locations but not habitats, but would have had to cross a different habitat to get to its new location.

Statistical analyses

In Chapter One I showed that gravid females used edges more than males and non-gravid females and in Chapter Two I showed that gravid females also thermoregulated more effectively and exploited their thermal environment more than males and non-gravid females. Therefore, in the present analyses I entered reproductive status and habitat as factors in two-way analyses of variance (ANOVA) to control statistically for the potential confounding effect of having gravid females thermoregulating more and also using certain habitats more than males and non-gravid females. Series of T_bs recorded from a single individual are not statistically independent. Therefore, all analyses were performed on data (T_b, d_e - d_b, or Ex) averaged for each individual over each active season. Some females changed reproductive status from one year to the next (gravid vs. non-gravid). Thus, I considered T_bs measured for an individual in different years to be independent. The tracking periods for individual snakes were different and I did not have

complete T_b data for each individual. Therefore, it was not practical here to use repeated measure analyses on individuals.

I performed all analyses using JMP Version 3.2 (SAS Institute 1997) on a Macintosh desktop computer. I inspected Box plots to determine if the assumptions of normality and homogeneity of variance were upheld. I detected no significant violations of these assumptions. I accepted significance of statistical tests at $\alpha = 0.05$, but I discuss marginally non-significant results when I deem them to be important. I report all means ± one standard error unless otherwise mentioned.

Results

During the three active seasons of the study (1 May to 30 September of 1997 to 1999), I recorded 130,669 T_bs from the 53 individuals I followed. For further analyses, I reduced these readings to 29,722 hourly mean T_bs. Of these hourly mean T_bs, the habitat occupied by the snake was known for 19,884 (66.9%) of the one-hour intervals. Of these hourly mean T_bs in known habitats, 11,764 (59.2%) were recorded during the day (600h - 1800h) and 8,120 (40.8%) during the night.

Thermal quality of habitats

For each hour of the entire active season I calculated a mean T_e for each habitat available to black rat snakes. T_e measurements in each habitat

indicated that, on average, T_e did not reach the lower bound of T_{set} in any of the shaded habitats (forest, barn, under flat rocks) during the course of the day (Fig. 3-1). Forests had the lowest mean T_e (Table 3-1) and were the coolest habitat for most of the day. In all exposed habitats (rock outcrops and field/wetland), T_e exceeded the upper bound of T_{set} daily from 900h to 1830h on average (Fig. 3-1). The two retreat sites in which I measured T_es (barn and under flat rocks) were buffered from daily climatic variation. Retreat sites tended to be warmer than other habitats during the night and cooler during the day.

I used the mean d_e calculated for the entire active season to measure the average thermal quality of the habitats available to black rat snakes. Mean d_es were highest for the open habitats (field/wetlands and rock outcrops), intermediate for forests, and lowest for the two retreat sites (barns and under flat rocks) and for the three types of edges (Table 3-1). Open habitats were too cold at night and too warm during the day, forests were never too warm but often too cold, and retreat sites were buffered from the daily variations in T_e but were slightly too cold. However, because I have assumed that edge habitats allowed simultaneous access to the high T_es of the open habitat and low T_es of the forest during the daytime, snakes in edges would have the potential to maintain T_bs very close to T_{set}. I also calculated the proportion of time that d_e equalled zero in each habitat (Table 3-1). Values of $d_e = 0$ occurred three to five times more often in the three edge habitats than in other

habitats. Overall these results indicate that, from a thermoregulatory perspective, habitat edges are superior to all other habitats. Edges were followed in thermal quality by retreat sites, then by forest, and finally by the open habitats.

Body temperatures

For the analyses of the thermoregulatory behaviour of black rat snakes while in different habitats, I grouped the different edge types and eliminated the habitats for which I had fewer than 100 hourly mean T_bs recorded. This produced three habitat categories: (1) inside barns, (2) in edge habitats, and (3) in the forest. This classification scheme was necessary to reduce the number of classes for the habitat factor and thereby increase the power and meaningfulness of the ANOVAs. Black rat snakes maintained higher mean T_bs during the day than during the night in all three habitats, and their mean T_bs were always lower than the lower bound of their T_{set} except in barns during the day. However, although black rat snakes experienced smaller mean d_bs during the day than during the night in edges and forest (as one would expect based on their mean T_bs), d_bs were lower at night in barns. This is due to the fact that, during the day in barns, black rat snakes sometimes maintained T_bs that were actually above their T_{set}, which happened rarely in edges and almost never in forest. The percentage of d_bs = 0 was higher during

the day than during the night in edges and forest, but equal in barns, again because some T_bs were actually above T_{set} in barns during the day.

I used two-way ANOVAs (with reproductive status included as a control variable) to test whether the T_bs, d_bs, and the percentage of d_bs = 0 of black rat snakes differed while they were in barns, edges, and forest during the day (based on 11,764 hourly mean T_bs) and during the night (based on 8,120 hourly mean T_bs). ANOVA showed that black rat snakes maintained significantly different T_bs while in the three habitat types during the day ($F_{(2,163)} = 7.231$, $p = 0.001$) and during the night ($F_{(2,93)} = 7.872$, $p < 0.001$). Tukey-Kramer HSD tests indicated that rat snakes had significantly higher T_bs while in barns than while in edges and significantly higher T_bs while in edges than while in forest during the day. During the night, black rat snakes had significantly higher T_bs while in barns than while in other habitats, but their T_bs were not significantly different while in edges and forest (Fig. 3-2).

ANOVA also indicated that black rat snakes experienced deviations of their T_bs from their T_{set} (d_bs) that were significantly different in the three habitats during the day ($F_{(2,163)} = 5.849$, $p = 0.004$) and during the night ($F_{(2,93)} = 7.486$, $p = 0.001$). Tukey-Kramer HSD tests indicated that, during both day and night, rat snakes had d_bs that were significantly smaller while in barns than while in the other two habitats and smaller, but not significantly so, while in edges than while in the forest (Fig. 3-2).

Black rat snakes spent a significantly different proportion of time with T_bs within their T_{set} (d_b = 0) while in the three habitats during the day ($F_{(2,163)}$ = 3.784, p = 0.025) and during the night ($F_{(2,93)}$ = 8.592, p < 0.001). Tukey-Kramer HSD tests revealed that during the day black rat snakes spent a significantly higher percentage of time with T_bs within T_{set} while in barns than while in forest. However, the percentage of time that rat snakes spent with T_bs within T_{set} was not significantly different while they were in edges and while they were in barns and forest. During the night, black rat snakes spent a significantly higher percentage of time with T_bs within T_{set} while they were in barns than while they were in edges and forest (Fig. 3-2). Therefore, collectively these results indicate that, from a purely thermoregulatory perspective, black rat snakes should have spent their time in barns because this was the habitat where they experienced the smallest deviations of T_bs from their T_{set}.

Effectiveness of thermoregulation

I used a two-way ANOVA (with reproductive status included as a control variable) to test whether black rat snakes had indices of thermoregulation effectiveness (d_e - d_b) that differed while they were in barns, edges, and forest during the day (based on 11,764 hourly mean T_bs) and during the night (based on 8,120 hourly mean T_bs). Black rat snakes had

higher indices of thermoregulation effectiveness during the night than during the day in all habitats.

In relation to the thermal opportunities offered within each habitat, black rat snakes had indices of thermoregulation effectiveness (d_e - d_b) that differed significantly by habitat during the day ($F_{(2,163)} = 31.581$, $p < 0.001$) but not during the night ($F_{(2,93)} = 0.055$, $p = 0.95$). Tukey-Kramer HSD tests indicated that black rat snakes thermoregulated significantly less effectively while in edges than while in barns and forest during the day (Fig. 3-3). This indicates that while in edges, snakes were not always seeking the best microhabitats from a thermoregulation perspective. For example, rat snakes could have been spending more time in the shade on the forest side of the edge rather than closer to the open habitat (field/wetland or rock outcrop) where T_es within their T_{set} were available. It should be emphasised that these results are driven both by differences in the T_bs black rat snakes maintained while in the different habitats and by differences in the thermoregulatory opportunities (T_es) available in each habitat. Therefore, the results are truly a measure of how much effort rat snakes put in thermoregulation while in the different habitats because they quantify how much snake T_bs departed from the average T_es for all microhabitats in the habitat and, thus, how selective they were about their microhabitat while in this habitat. While in edges, the deviations of T_bs from T_{set} (d_bs) that black rat snakes experienced were actually larger than the average deviations of T_es from T_{set} (d_es) indicated by

the models in edges, whereas while rat snakes were in barns and forest their mean d_bs were larger than the mean d_e for the habitat. Thus, black rat snakes were not selective of their microhabitat while in edges (in fact, the microhabitats they chose had a mean thermal quality below the average for edges), and they were somewhat selective while in barns and forest (the microhabitats they chose had a mean thermal quality above the average for the habitat).

Thermal exploitation

Of the 19,884 hourly mean T_bs recorded from black rat snakes in known habitats, the T_es indicated that it would have been possible for a snake to achieve T_bs within T_{set} in the habitat in which it was located for a total of 4,865 hourly mean T_bs (4,523 hourly mean T_bs during the day and 342 hourly mean T_bs during the night). Again, black rat snakes tended to have higher indices of thermal exploitation during the night than during the day in all habitats.

Two-way ANOVAs (controlling for the reproductive status of individuals) testing for differences in the extent to which black rat snakes exploited their thermal opportunities (Ex) between habitats, in relation to the thermal opportunities offered by each habitat, revealed a significant interaction between reproductive group and habitat during the day (reproductive group*habitat $F_{(4,139)} = 2.757$, $p = 0.030$) and during the night (reproductive group*habitat $F_{(4,37)} = 3.024$, $p = 0.030$). Separate one-way

ANOVAs for each reproductive group indicated that, during the day, the extent to which black rat snakes exploited their thermal opportunities (Ex) differed by habitat for males ($F_{(2,42)} = 6.562$, $p = 0.003$) and non-gravid females ($F_{(2,60)} = 7.306$, $p = 0.001$), but not for gravid females ($F_{(2,37)} = 0.396$, $p = 0.676$). Tukey-Kramer HSD tests showed that male black rat snakes exploited their thermal opportunities significantly more while in barns than while in edges and forest. However, non-gravid female black rat snakes exploited their thermal opportunities significantly less while in edges than while in barns and forest (Fig. 3-4). During the night, the extent to which black rat snakes exploited their thermal opportunities (Ex) differed by habitat for non-gravid females ($F_{(2,17)} = 4.489$, $p = 0.027$), but not for males ($F_{(2,10)} = 1.105$, $p = 0.368$) or gravid females ($F_{(2,10)} = 1.583$, $p = 0.253$). Post-hoc tests indicated that non-gravid females exploited their thermal opportunities less while in edges than while in barns and forest (Fig. 3-4). However, the results of the analysis during the night should be interpreted cautiously because they are based on only 342 hourly mean T_bs.

Discussion

For black rat snakes, the environment in eastern Ontario is thermally challenging. Only open habitats that received full solar radiation throughout the day (fields/wetlands and rock outcrops) offered T_es that, on average, rose above the lower bound of T_{set} during the course of the day. Most of the

terrestrial habitat in the study area is forested (48%, Chapter One) and this was the coolest of all the habitats available to black rat snakes. T_es in the forest were above the lower bound of T_{set} in only ≈ 7% of the observations. Thus, despite being a forest species, a rat snake in Ontario usually has to use habitats other than forests or has to use forest edges to be able to achieve T_bs within its T_{set}.

Based on the strong preference for edges by black rat snakes described in Chapter One, I had hypothesised that edges should be of higher thermal quality than the other habitats in the study area. I confirmed that edges were the habitats with the highest thermal quality. Edges were followed in thermal quality by retreat sites and then by forest. Edges had among the lowest mean d_es of all the habitats and had the highest proportions of d_es equal to 0. The thermal quality of edges is conferred by their location at the interface of the coolest habitat (forests) that is always shaded and the warmest habitats (open habitats: fields/wetlands and rock outcrops) that receive full solar radiation. This result is a consequence of my assumption that snakes in edges always have access both to a refuge from high temperatures (forest) and to the warmest possible habitats (open habitats). However, the assumption seems reasonable because there is almost no travel time involved in shuttling between these two extremes. Thus, edges provide the best opportunities for behavioural thermoregulation of all the habitats in the study area.

The different thermoregulation metrics revealed consistent differences in how black rat snakes thermoregulated in the different habitats. Black rat snakes tended to maintain higher T_bs and tended to have T_bs closer to, or more often within, T_{set} while in barns than while in edges and while in edges than while in forest. Overall, these results indicate that black rat snakes experienced more favourable T_bs while in barns than while in edges and while in edges than while in forest. Therefore, my prediction that snakes should experience more favourable T_bs in edges than in other habitats was only partially supported. However, it should be noted that in totally pristine environments, barns would not be available and black rat snakes would experience the most favourable T_bs while in smaller natural retreats such as under flat rocks on rock outcrops (these retreats are most often situated in edges because they are within 15 m of the forest) and while in edges. Thus, among the natural habitats, black rat snakes do experience more favourable T_bs while in edges.

When I considered the thermoregulation effectiveness of black rat snakes with regard to the thermal opportunities offered by each specific habitat, I found almost no differences between habitats during the night. However, during the day black rat snakes thermoregulated much less effectively while in edges than while in barns and forest. In fact, my calculations of thermoregulation effectiveness in edges during the day showed that black rat snakes were actually using thermally favourable

microhabitats less than their availability (negative $d_e - d_b$). The differences between habitats were more variable for thermal exploitation (Ex) in relation to the thermal opportunities offered by each habitat, partly due to significant interactions between habitat and reproductive status. The trend was for black rat snakes to exploit their thermal opportunities more while in barns and less while in edges during the day. During the night there was no consistent trend and small sample sizes made the validity of the comparisons questionable. Overall these results indicated that the snakes did not necessarily achieve more favourable T_bs in the thermally superior habitats by thermoregulating more in those habitats. In fact, while in edges, the snakes appeared to invest less in thermoregulation but nonetheless benefited from the high thermal quality of edges. In contrast, while in forest during the day, black rat snakes thermoregulated more but realised lower T_bs than while in edges. Even a small thermoregulatory effort in a habitat of high thermal quality can produce higher T_bs than a large thermoregulatory effort in a habitat of low thermal quality. Therefore, my prediction that black rat snakes should invest more in thermoregulation while in thermally superior habitats was not supported.

One potential criticism of how I have interpreted my results is that black rat snakes may not actively thermoregulate, but may simply be thermoconformers that favour edges for reasons other than thermoregulation and, therefore, their increased T_bs in edges as compared to

forest is simply a by-product of their habitat preference. For example, black rat snakes could be attracted to edges for foraging (Weatherhead and Charland 1985, Durner and Gates 1993) or for predator avoidance (Chapter One). Under this scenario, black rat snakes would tend to have higher T_bs in edges than in the forest simply because the T_es in edges are higher. Two lines of evidence suggest that black rat snakes are indeed actively thermoregulating in edges and that the higher T_bs recorded in those habitats compared to forest are not attained passively. First, when snakes are fed in a laboratory thermal gradient, they increase their T_bs following feeding (Slip and Shine 1988, Lutterschmidt and Reinert 1990). Similarly, they thermoregulate more effectively following feeding in the field (Beck 1996) and feeding in the field is associated with an increase in the use of edges (Chapter Four). Increased thermoregulation following feeding is expressed both in the laboratory and in the field and is therefore not simply a by-product of habitat preference. Second, gravid female snakes have been reported to thermoregulate more carefully than males and non-gravid females in the laboratory (Tu and Hutchison 1994), in semi-natural enclosures (Charland and Gregory 1990), and in the field (Charland and Gregory 1995, Brown and Weatherhead 2000). Gravid females have also been shown to favour the habitats that offer the best opportunities for thermoregulation (Reinert 1984a,b, Reinert and Zappalorti 1988). Because the increase in thermoregulation has been reported in the laboratory, in semi-natural settings, and in the field, we can infer that it is not a simple passive

consequence of habitat use and, further, that habitat use is the means by which increased thermoregulation by gravid females is achieved in the field.

There are at least two possible explanations for the lack of agreement I documented between thermal quality and the extent of thermoregulation. First, it is possible that my assumption that black rat snakes in edges have access to both sides of the boundary at no cost is wrong and that the unsuspected costs actually decrease the thermal quality of edges below that of retreats such as barns. However, even if my assumption is wrong, it would not explain why thermoregulatory effort was less in edges than in the forest, because the thermal quality of edges cannot be lower than the thermal quality of forest; it can only be equal or superior given that edges are half forest and therefore give access to forest and to an open habitat. Second, other costs of thermoregulation (besides time) could be at play and could differ between the habitats. For example, predation risk could be lower in retreats than in edges and therefore the costs associated with thermoregulating in edges may be higher than the costs of thermoregulation in barns. Alternatively, snakes in edges may be foraging as well as thermoregulating, changing the priority associated with thermoregulation. Also, perhaps black rat snakes invest more in thermoregulation in barns and forest than in edges because the thermal heterogeneity is less in the former habitats than in the latter. It may be easier for black rat snakes to evaluate their thermal options in a habitat of low

thermal heterogeneity than in a habitat of high thermal heterogeneity because not as much sampling is required in the former.

In the cost-benefit model of thermoregulation presented by Huey and Slatkin (1976), the cost of thermoregulation is believed to increase monotonically with the magnitude of the difference between the T_b to be achieved (for field-active ectotherms this T_b to be achieved is T_{set}) and T_es. Thus, as T_es are further from T_{set} (this is denoted by high d_es, indicative of low thermal quality), the cost of thermoregulation increases. The central prediction of their model relating the cost of thermoregulation to the extent of thermoregulation is that "For all reasonable forms for c (cost curve) and b (benefit curve), we would predict that a lower value of k (increased thermoregulation) would be optimal in the habitat with the lower cost (higher thermal quality)" (Huey and Slatkin 1976, p. 371). Black rat snakes in Ontario experience very challenging thermal conditions and I had therefore expected that they should be particularly sensitive to the thermal quality of habitats. Thus, the snakes should have thermoregulated more in habitats of high thermal quality because the cost of thermoconformity is high when the thermal quality of the environment is generally low (the T_bs experienced by a thermoconformer are far from T_{set}: Chapter Two). The mismatch I observed between thermoregulation in a habitat and the thermal quality of this habitat is contrary to the central prediction of the cost-benefit model of thermoregulation.

Although it seems reasonable to assume that the optimal amount of thermoregulation for an ectotherm is determined by some trade-off between costs and benefits, my results suggest that the main cost of thermoregulation identified in the original model is insufficient to explain the extent of thermoregulation in black rat snakes. It is likely that other factors expected to influence the extent of thermoregulation, such as foraging requirements and predation risks, affect the shape of the cost and benefit curves more than differences in the thermal quality of habitats. Two research needs are suggested. First, the cost-benefit model needs to be modified so that it specifically incorporates the influence of predation risk and foraging on thermoregulation. Second, and probably more challenging, will be to design field studies (both correlational and experimental) to test the predictions made by a model that is ecologically more complex than its predecessor.

Table 3-1: Mean ±1 SE, maximum, and minimum operative temperatures recorded in each habitat available to black rat snakes. Mean ±1 SE, maximum, and percent equal to zero deviations of operative temperatures from the preferred body temperature range of black rat snakes in each habitat in Ontario. Values for mean T_e and mean d_e are from Chapter Two.

Habitat	T_e			d_e		
	mean	max	min	mean	max	% = 0
Forest	17.5 ±0.06	34.5	-2.7	9.2 ±0.06	29.2	6.2
Field/Wetland	20.8 ±0.13	59.1	-7.9	12.0 ±0.07	34.4	4.6
Rock outcrop	25.9 ±0.13	63.6	-5.6	9.5 ±0.07	33.8	8.0
Barn	20.4 ±0.04	34.3	7.1	6.2 ±0.04	19.4	6.3
Under flat rock	21.5 ±0.05	35.6	3.1	5.5 ±0.04	23.4	12.5
Edge forest-field/wetland	-	-	-	7.5 ±0.06	29.2	29.5
Edge forest-rock outcrop	-	-	-	5.6 ±0.06	29.2	40.7
Edge forest- water body	-	-	-	7.0 ±0.06	29.2	32.7

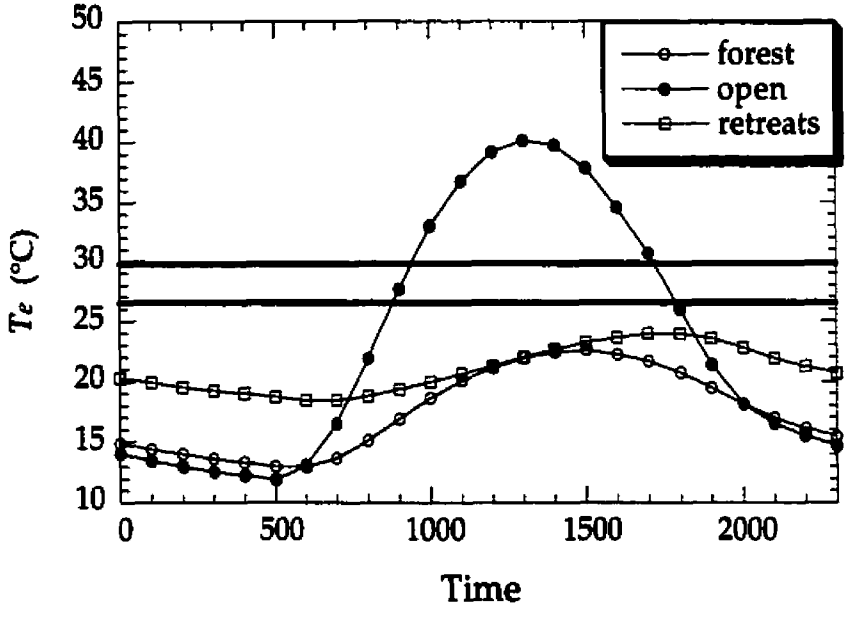

Fig. 3-1: Mean operative environmental temperatures (T_es averaged over the whole active season) for each hour of the day in forest, open habitats (mean of operative environmental temperatures in fields/wetlands and rock outcrops), and retreat sites (mean of operative environmental temperatures in barns and under flat rocks on rock outcrops) in eastern Ontario. The horizontal lines represent the preferred body temperature range (T_{set}) of black rat snakes in eastern Ontario.

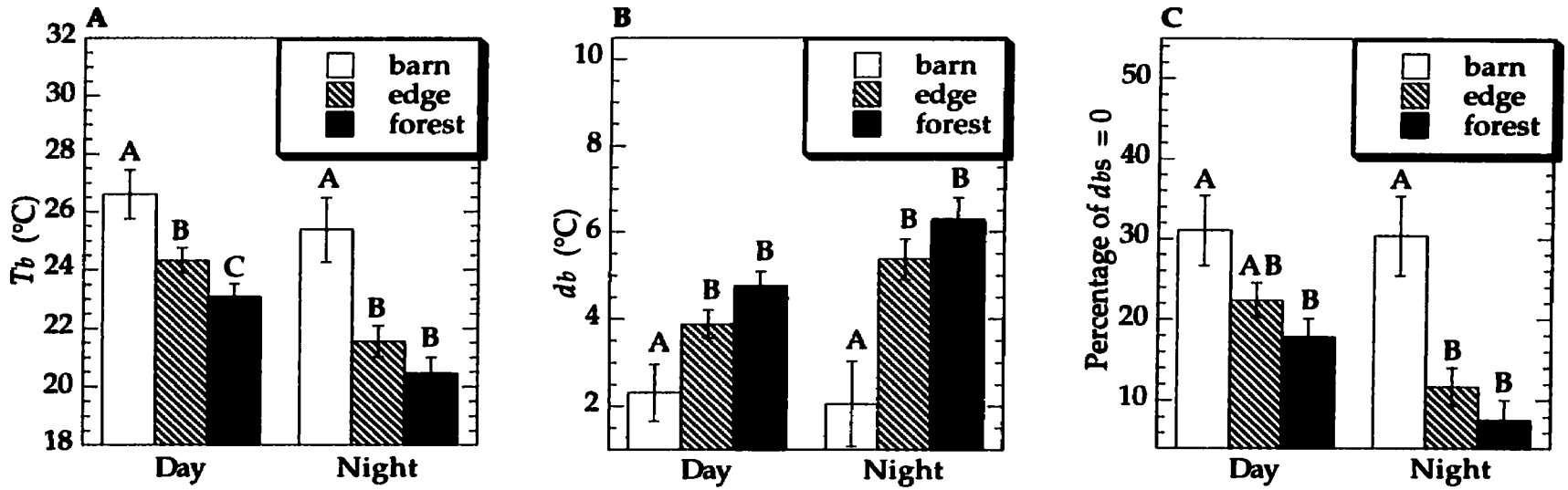

Fig. 3-2: Least square mean (corrected for reproductive status) body temperatures (T_bs) (A), deviations of body temperatures from the preferred body temperature range (d_bs) (B), and percentages of deviations of body temperatures from the preferred body temperature range (d_bs) equal to zero (C) in barns, edge habitats, and forest during the day and during the night for radio-tracked black rat snakes in eastern Ontario. Means with the same letters are deemed not significantly different based on Tukey-Kramer HSD tests.

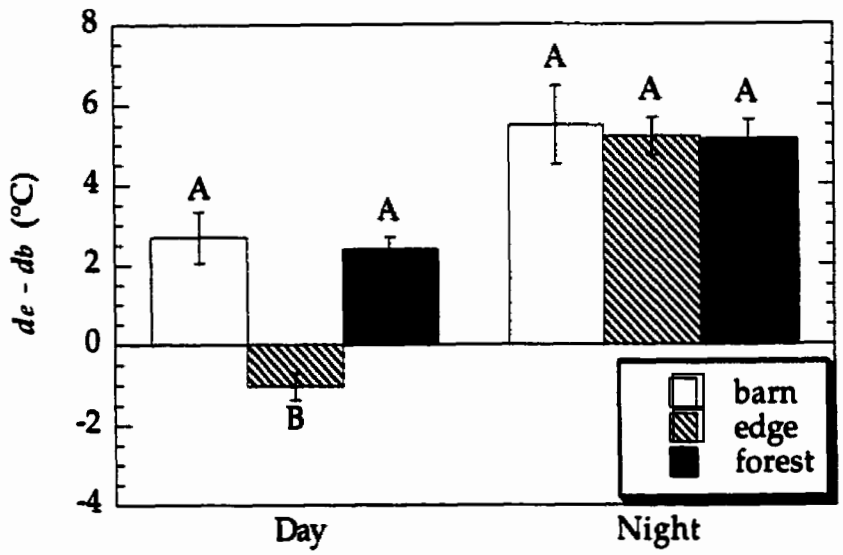

Fig. 3-3: Least square mean (corrected for reproductive status) thermoregulation effectiveness index ($d_e - d_b$) calculated in relation to the thermal opportunities offered by each separate habitat in barns, edge habitats, and forest during the day and during the night for radio-tracked black rat snakes in eastern Ontario. Means with the same letters are deemed not significantly different based on Tukey-Kramer HSD tests.

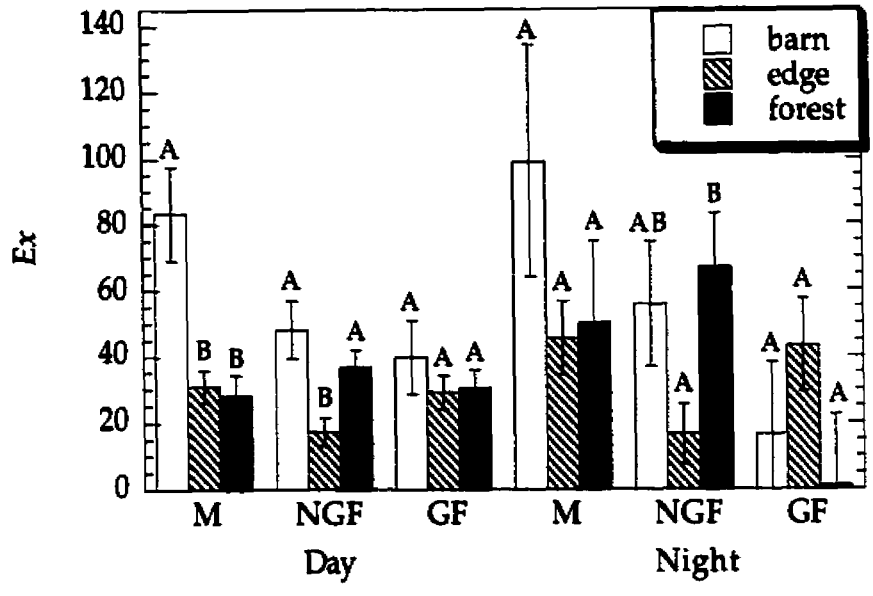

Fig. 3-4: Least square mean (corrected for reproductive status) thermal exploitation index (*Ex*) calculated in relation to the thermal opportunities offered by each separate habitat (index calculated separately for males (M), non-gravid females (NGF), and gravid females (GF)) in barns, edge habitats, and forest during the day and during the night for radio-tracked black rat snakes in eastern Ontario. Means with the same letters are deemed not significantly different based on Tukey-Kramer HSD tests.

CHAPTER FOUR

An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes

Introduction

All physiological processes are temperature dependent. Thus, because reptiles are ectotherms, variation in their body temperature (T_b) affects their developmental, physiological, and behavioural processes (Christian and Tracy 1981, Hertz et al. 1982, Arnold and Bennett 1984). Hence, virtually all aspects of reptile ecology are affected by T_b and T_b ultimately has a significant impact on fitness (Huey and Kingsolver 1989). All ectotherms obtain heat from their environment (Pough 1980) and T_b regulation is achieved behaviourally by adjusting habitat selection, body posture, and timing of activity (Huey et al. 1989, Grant 1990). Thermoregulation is probably the single most important proximate factor influencing habitat selection by terrestrial squamates (Reinert 1993). My general goal in this chapter was to investigate the association between foraging and thermoregulation in black rat snakes as a further step in understanding the relationship between habitat use and thermoregulation in snakes.

The thermoregulatory behaviour of snakes following feeding provides an ideal opportunity to study thermal ecology. Snakes typically ingest large meals at infrequent intervals and several factors should make hastened digestion through increased thermoregulation advantageous in this situation. (1) A large meal might impair locomotion, making the snake more susceptible to predators. (2) Time spent inactive while digesting cannot be

spent doing other more profitable activities, such as searching for mates or the next meal (Greenwald and Kanter 1979). (3) Slow digestion allows decomposition of the prey item inside the snake, leading to regurgitation of the meal or even death (Naulleau 1983). From a practical perspective, thermoregulatory behaviour following feeding is easily studied because it has a precisely defined start time, unlike other factors that have been reported to increase thermoregulatory behaviour such as reproduction (Charland and Gregory 1990, Brown and Weatherhead 2000, Chapter Two) or skin shedding (Gibson et al. 1989). Also unlike other factors, the association between feeding and thermoregulation is amenable to experimentation through supplemental feeding by the researcher (Brown and Weatherhead 2000).

Although several researchers have recognised the experimental potential of thermoregulation following feeding, exploitation of that potential has been largely limited to studies of captive animals. Postprandial thermophily (PPT) in the laboratory has been reported in many snake species (Regal 1966, McGinnis and Moore 1969, Lysenko and Gillis 1980, Bozinovic and Rosenmann 1988, Slip and Shine 1988, Gibson et al. 1989, Touzeau and Sievert 1993), but may not be important in others (Kitchell 1969, Lysenko and Gillis 1980, Hammerson 1989, Tu and Hutchison 1995). There is obviously no consensus about the generality of the phenomenon in snakes (Lilywhite 1987), and it is unclear whether this reflects real differences among species or is an artefact of studying captive animals. Only two studies of PPT have been

conducted on free-living snakes (Beck 1996, Brown and Weatherhead 2000), and only Brown and Weatherhead (2000) simultaneously tested for the presence of PPT in the laboratory and in the field. They found that northern water snakes (*Nerodia sipedon*) did not significantly increase their T_b, and did not thermoregulate more carefully following feeding in either the laboratory or the field. However, their study also revealed that environmental operative temperatures (T_es) within the preferred T_b range (T_{set}) of water snakes were widely available in the snakes' habitat, thus making it very easy for the snakes to maintain their preferred T_b. To assess the generality and importance of PPT in the wild, we need information on the postprandial thermoregulatory behaviour of species that face thermally challenging environments, such as black rat snakes in eastern Ontario (see Chapter Two). In addition, I am not aware of any study that has formally examined habitat selection in relation to digestion of a meal in snakes, despite the tenet that habitat selection is one of the primary ways in which snakes adjust their T_b (Reinert 1993). Thus, it is important not only to study PPT in free-living snakes, but also to document the role that habitat selection plays in this phenomenon.

I had three specific objectives in this chapter. First, I tested the prediction that black rat snakes elevate their T_b following a meal in a laboratory thermal gradient. Second, I tested the prediction that free-ranging rat snakes increase T_bs and/or increase the extent of behavioural thermoregulation following a meal. Third, I tested the prediction that free-

ranging black rat snakes use habitat selection as the mechanism for behavioural thermoregulation.

Although feeding is amenable to experimentation, several logistical problems need to be overcome and a complicated design is required to conduct this seemingly simple experiment. First, because snakes are shy and elusive animals, opportunities for experimental feeding are scarce (e.g., I was able to observe radio-implanted rat snakes in only 30% of 3715 daily relocations, Chapter One), so an extensive radio-telemetry study is required. Second, one needs to obtain natural prey items of the study species and entice both captive and free-ranging snakes to voluntarily ingest them (force-feeding is not an option because the associated trauma could lead to abnormal behaviour). Third, it is necessary to record T_bs and habitat use for at least 24 hrs prior to and following feeding, even if the snakes move following feeding. Obtaining data on T_b and habitat use prior to feeding allows individual snakes to be used as their own controls. Fourth, in addition to monitoring T_bs, one needs to monitor simultaneously T_es in all habitats available to the study species prior to and following feeding to allow calculation of thermoregulatory indices (Hertz et al. 1993, Christian and Weavers 1996, Chapter Two). Finally, one needs to repeat the feeding trials on a sufficient number of individuals to achieve adequate statistical power. The combination of these difficulties probably explains why this experiment has not been performed previously.

Materials and Methods

Study area and study animals

I conducted this study from 1997 to 1999 at the Queen's University Biological Station, approximately 40 km north of Kingston (Ontario, Canada). The study area was approximately 9.5 km by 2.5 km along the north-western shore of Lake Opinicon and was mostly covered by second-growth deciduous forest (Chapter One). Black rat snakes were captured both at communal hibernacula during spring emergence (Blouin-Demers et al. 2000b) and opportunistically during the active season (Prior et al. 2001). I selected a subset of individuals and surgically implanted temperature sensitive radio-transmitters (Model SI-2T, Holohil Systems Inc., Carp, Ontario) in them under isoflurane anaesthesia (Appendix One). Transmitters weighed 8.6 g (maximum ratio of transmitter mass : body mass = 0.025) and had 20 months battery life at 20°C. Calibration curves relating transmitter pulse rate to temperature were supplied by the manufacturer, but I verified the accuracy of the curves (Chapter Two). I used two automated radio-telemetry data loggers (SRX 400, Lotek Engineering Inc., Newmarket, Ontario) to record T_bs of black rat snakes every 10 min. I then averaged T_bs for each individual each hour. From May 1997 to November 1999 I followed 17 males and 36 females for periods ranging from 1 to 30 months. Twenty-three snakes (nine males and 14

females) were followed in multiple years and therefore I have data for 79 "snake years" (25 "male years" and 54 "female years").

Feeding trials

To determine if PPT occurred in black rat snakes, I needed to feed radio-implanted snakes because very few natural feeding events are observed (G. Blouin-Demers, personal observation). I fed rat snakes by offering them a euthanised prey item clipped to the end of an extendable pole. I fed snakes in this manner in the laboratory and in the field (see below). During each trial, I fed the snakes as much food as they would voluntarily ingest. Although initially hesitant, the snakes soon learned to recognise this feeding opportunity and started taking food directly from my hands. The rodents were weighed prior to being fed to snakes and I was therefore able to calculate the total amount of food consumed by the individual for each feeding trial. Snakes were always fed at least 10% of their fasted body weight. When attempting feeding in the field, I could not tell if the snake had fed recently, except if it had an obvious food bulge. However, I know that the snakes had substantially more food in their stomach after I fed them than before, and that they must have been hungry at the time of feeding if they accepted food. I ensured that snakes had an empty stomach prior to feeding in the thermal preference chamber by fasting them for a week before testing. Feeding trials were conducted at least six weeks after radio-transmitter implantation to allow ample time for healing.

PPT in the laboratory

I tested for PPT in the laboratory using a thermal gradient chamber (Chapter Two). I introduced radio-implanted rat snakes individually in the chamber under two treatments: post-absorptive and recently fed (see above). The order in which the treatments were administered was randomised and snakes were given 24 hrs to acclimatise to the setting in each case. I then recorded T_bs selected in the gradient every 10 min for 24 hrs using a telemetry data logger. I compared the bounds of the central 50% of observed T_bs (Hertz et al. 1993, Christian and Weavers 1996) and the mean T_b selected for each individual under both treatments to determine if T_b selection varied following feeding.

PPT in the field

To determine if PPT occurred in the field, I fed free-ranging radio-implanted black rat snakes and compared their thermoregulatory behaviour for 24 hrs preceding feeding to their thermoregulatory behaviour for 24 hrs following feeding. Several methods have been designed to quantify the thermal behaviour of ectotherms and I used three different indices of thermoregulation in my analyses. The simplest method I used was to calculate the average T_b maintained by snakes before and after feeding. I also used more refined thermoregulation indices that compare the extent to which a species actually experiences T_bs within its T_{set} (the "accuracy" of T_b)

to the extent to which the habitat where the species lives allows T_bs within the T_{set} to be reached (the "thermal quality" of the habitat). From the measures of the accuracy of T_b (d_b) and of the thermal quality of habitats (d_e), Hertz et al. (1993) derived an index of the effectiveness of thermoregulation $E = 1 - (\bar{d}_b / \bar{d}_e)$. In Chapter Two I showed that d_e - d_b was a better index of thermoregulatory effectiveness than E because it does not use a ratio. I therefore used this latter index in my analyses. Finally, I used an additional index of thermoregulation (Ex) developed by Christian and Weavers (1996) that determines the extent to which animals exploit their thermal environment. Ex is the amount of time an animal spends within its T_{set} expressed as a percentage of the time when T_bs within its T_{set} were available in the habitat (as indicated by the T_es). I calculated all indices of thermoregulation for the whole day and for the daytime only (600h to 1800h) because I showed in Chapter Two that T_bs within T_{set} could rarely be achieved at night in my study area and that differences in thermoregulation between different snake groups were most pronounced during the day.

Habitat use before and after feeding

In Chapter One I showed that black rat snakes were more likely to use habitat edges than expected by chance. This preference was detected both by using a Multivariate Analysis of Variance and associated Discriminant Function Analysis on a large set of habitat variables and by simply classifying

the snake and random locations into habitat categories. In the latter analysis, I considered that a snake was in an edge when it was within 15 m of the boundary between the forest and an open habitat such as old hayfields or rock outcrops. I used the same working definition in the present chapter and simply classified snake locations prior to feeding and following feeding as being in an edge or not in an edge based on the 15 m criterion. I also compared the behaviour of experimentally fed snakes to the behaviour of snakes radio-tracked as part of a larger telemetry study (Chapter One and Chapter Two). Specifically, I compared their behaviour at relocation (classified as basking/resting or other) and their frequency of movement (where no movement was defined as a displacement ≤ 5 m) and predicted that (1) snakes should bask more following feeding and that (2) if edges are used for thermoregulation, snakes should travel more following feeding in the forest than following feeding in an edge. Finally, I analysed whether or not snakes moved following feeding was related to the daily maximum temperature (in °C) or the daily maximum solar radiation (in kW/m^2) because it should be more critical for snakes to reach a good thermoregulation site when environmental conditions are challenging.

Statistical analyses

Series of T_bs recorded from a single individual are not independent. Therefore, all analyses were performed on data (T_b, d_e - d_b, or Ex) averaged for each individual over the period before and after feeding. I used paired t-tests

(one-tailed because I was testing the hypothesis that snakes thermoregulate more carefully following feeding) to compare the thermoregulatory behaviour of individuals prior to and following feeding, except when comparing mean T_b where I used ANCOVA to control for the effect of measuring T_bs under different T_es. I compared the habitat use prior to and following feeding using a binomial test (Zar 1984). I compared the behaviour of snakes and the frequency of movements using Chi-square tests. I analysed the relationship between movement following feeding and weather using logistic regression.

The analyses were conducted on JMP Version 3.2 (SAS Institute 1997) and G•Power Version 2.1 (Buchner et al. 1997) on a Macintosh desktop computer. I inspected Box plots to determine if the assumptions of normality and homogeneity of variance were upheld. The only significant departure from these assumptions was that the frequency distribution of distances moved was non-normal. A square root transformation corrected the problem. All means are reported ± one standard error unless otherwise stated.

Results

PPT in the laboratory

Thirteen individual snakes were fed an average of 101.6 g ± 8.0 of food in the thermal preference chamber. This mean meal size represents 17.3% ± 1.9 of the snakes' mean body weight. I recorded 3,744 T_bs that were reduced to

624 individual hourly mean T_bs from these 13 snakes. I compared their T_b selection for 24 hrs while fasted to their T_b selection for 24 hrs following a large meal. Paired t-tests revealed that, after feeding, snakes had significantly higher mean T_bs and 25% quartiles, but had 75% quartiles that were not significantly different at $\alpha = 0.05$ (Table 4-1). Thus, black rat snakes exhibited postprandial thermophily in the laboratory and shifted the lower end of their T_{set} range (the 25% quartile) upward after feeding, which affected the mean T_b selected, but the upper end of the T_{set} range remained unchanged.

PPT in the field

I fed 23 individuals an average of 132.3 g ± 8.9 of food a total of 37 times in the field. This mean meal size represented 19.6% ± 1.4 of the snakes' mean body weight. However, because of the numerous difficulties in obtaining complete hourly T_b profiles from individual snakes (see above) and because the automated data-loggers were also used in a larger thermoregulation study (Chapter Two and Chapter Three), I only had complete data (≥ 90% complete) for 10 feeding events on eight individuals. This reduced data set consisted of 2,052 T_b readings averaged to 355 individual hourly mean T_bs. The following analyses of the thermoregulatory behaviour of free-ranging black rat snakes prior to and following feeding are based on the reduced data set. In Chapter Two, I used data from 41 post-absorptive individuals in the thermal preference chamber to determine that T_{set} when not digesting for the

population was 26.5°C to 29.8°C. I used these T_{set} values in my calculations of the thermoregulation indices.

For each of the eight snakes, I calculated a mean T_b, T_e, d_b, d_e, and Ex (for the whole day and for the daytime only) for the 24 hrs prior to feeding and the 24 hrs following feeding. To test whether or not snakes increase their T_b following feeding in the field, I used mean T_e and feeding status as independent variables and mean T_b as dependent variable in an ANCOVA. Least square mean T_bs (corrected for T_es) of rat snakes following feeding for the whole day or daytime only were not significantly different from T_bs maintained prior to feeding at $\alpha = 0.05$ (Table 4-2). Even if snakes do not elevate their T_b following feeding in the field, it is still possible they thermoregulate more carefully. Following the recommendations for the study of thermoregulation I presented in Chapter Two, I calculated two additional thermoregulation indices. I found that both indices of thermoregulation were higher following feeding. On average for the whole day or daytime only, the index $d_e - d_b$ was ≈ 1°C higher following feeding and Ex was ≈ 18% higher following feeding. However, paired t-tests indicated that none of the differences was statistically significant at $\alpha = 0.05$ (Table 4-2).

One problem with the t-tests I used to assess statistical significance of thermoregulation before and after feeding in the field is that, despite large effect sizes (all ds were ≥ 0.75 (Cohen 1977)), the power ($1 - \beta$) was very low

because of small sample sizes (Stevens 1996). Post-hoc power analyses indicated that power was ≤ 0.25 for all tests and that a sample size of ≥ 55 would have been necessary to achieve acceptable power (≈ 0.80: Stevens 1996). However, it is highly unlikely that such a considerable data set will become available in the near future given the difficulties involved in collecting this type of data (see Introduction). Under conditions of large effect sizes and low power due to small sample sizes, Cohen (1977) and Stevens (1996) have suggested adopting a more lenient α level such as 0.10 or 0.15 to improve power sharply. A more lenient α level is especially appropriate if the consequences of making a Type I error are not serious (Stevens 1996), such as in the present case. If I use $\alpha = 0.15$ in my analyses, several effects are deemed significant ($d_e - d_b$ whole day and *Ex* day only: Table 4-2).

Habitat use and movement by snakes before and after feeding

I fed 23 free-ranging individuals a total of 37 times (23 times in edges and 14 times in the forest). For the purpose of the present analysis, I considered that a snake was faced with a binomial choice following feeding: to be in an edge or not. After being fed in an edge (23 times), snakes were relocated in an edge the following day 21 times (Fig. 4-1). This result deviates significantly from random ($p < 0.001$) based on the proportions of the binomial distribution (Zar 1984). After being fed in the forest 14 times, snakes were relocated in an edge the following day 11 times (Fig. 4-1). This again was

more often than expected by chance ($p = 0.022$). In addition, snakes travelled shorter distances between feeding and relocation the following day when fed in edges (mean = 78.4 m ± 19.4) than when fed in the forest (mean = 139.2 m ± 24.8; $t_{(35)} = 1.931$), $p = 0.06$). When I considered movement as simply whether or not the snakes moved ("not moved" ≤ 5 m between relocations and "moved" > 5 m between relocations), the effect of habitat is more obvious. Snakes were significantly more likely to move when fed in the forest than when fed in edges ($N = 37$, $\chi^2_{(1)} = 7.63$, $p = 0.006$). In fact, snakes fed in the forest moved > 5 m between relocations in 100% of trials compared to only 69.6% for snakes fed in edges. Logistic regression indicated that whether or not snakes moved following the day of feeding was not influenced by maximum temperature ($N = 37$, Wald $\chi^2_{(1)} = 0.13$, $p = 0.72$) or by maximum solar radiation ($N = 37$, Wald $\chi^2_{(1)} = 0.14$, $p = 0.70$) during that day. When comparing experimentally fed snakes to snakes followed during the same time period as part of a larger telemetry study (Chapter One), I found that, following feeding, snakes were significantly more likely to be found basking ($N = 1353$, $\chi^2_{(1)} = 4.75$, $p = 0.029$) and significantly more likely to have moved ($N = 1353$, $\chi^2_{(1)} = 18.21$, $p < 0.001$).

Discussion

I found that black rat snakes exhibited PPT in the laboratory. In the field, rat snakes did not elevate their T_b following feeding, but I found evidence they thermoregulated more carefully subsequent to a meal and that habitat selection was the means by which more careful thermoregulation was achieved.

Two studies have investigated PPT in free-ranging snakes (Beck 1996, Brown and Weatherhead 2000), and only Brown and Weatherhead (2000) investigated PPT simultaneously in the field and in the laboratory using the same study population. Therefore, it is not possible to determine the extent to which my results on the thermoregulatory behaviour of free-ranging black rat snakes following a meal are typical of other species. Consistent with the absence of PPT in water snakes in a laboratory thermal gradient, Brown and Weatherhead (2000) did not detect PPT or more careful thermoregulation following feeding by water snakes in the field. I found similar consistency between behaviour in the laboratory thermal gradient and in the field in black rat snakes. However, unlike water snakes, rat snakes thermoregulated more carefully following a large meal. Thus, the limited data available to date suggest that the response of snakes following feeding in a laboratory thermal gradient is a reliable predictor of their response to feeding in the wild. Therefore, it is possible to use studies with captive snakes to address the question of whether any ecological factors consistently differentiate snakes

that exhibit PPT from those that do not. Several potential determinants have been proposed, such as the size and frequency of meals (Touzeau and Sievert 1993), the type of food ingested, or the thermal environment faced by the species.

A survey of the literature revealed 13 studies (including the present one) of PPT in captive snakes (summarised in Table 4-3). The first surprising fact is that most of these studies have been cited in snake thermal ecology review articles (e.g., Lillywhite 1987, Peterson et al. 1993) as evidence for PPT (or lack thereof), despite that only four were based on more than five animals (Table 4-3). In studies with such small sample sizes, the probability that the results will generalise to the population is small (Stevens 1996). Another surprising fact is that in seven studies, the T_bs of snakes were measured using techniques that are likely to affect the snakes' behaviour, such as cloacal and gastric probes taped to the animal or large ingested transmitters. Ingested transmitters have actually been demonstrated to induce thermophily in some snakes (Lutterschmidt and Reinert 1990).

Given that many previous studies of PPT in the laboratory suffer from low sample sizes and use of disruptive techniques, it does not seem possible to draw strong conclusions regarding the determinants of PPT at the present time, especially given that some results appear contradictory. For example, Lutterschmidt and Reinert (1990) and Brown and Weatherhead (2000) studied approximately the same number of *Nerodia sipedon* (five and seven,

respectively) fed the same prey (fish) and both used non-invasive T_b collection methods, yet, PPT was found by Lutterschmidt and Reinert (1990) and not by Brown and Weatherhead (2000). One difference between the two studies is that Brown and Weatherhead's used a longer acclimation and testing period (12 and 12 hrs, respectively) than Lutterschmidt and Reinert (2 and 4 hrs, respectively).

If we consider only the five studies where researchers used at least five study subjects and non-invasive T_b collection methods (i.e., Slip and Shine 1988, Gibson et al. 1989, Lutterschmidt and Reinert 1990, Brown and Weatherhead 2000, this study), four found evidence of PPT and a single one did not (Table 4-3). Within the studies that found evidence of PPT, two species were fed rodents and two fish. Therefore, it appears that PPT occurs in the majority (4 / 5) of species for which an adequate data set exists, but it does not seem that food type influences the presence of PPT. Studies of PPT with enough subjects of other species will be required before we can assess whether PPT varies along taxonomic lines or simply varies from population to population according to the local optimal tradeoffs between the costs (e.g., time) and benefits (e.g., hastened digestion) of PPT.

Snakes fed in the field ate more (mean = 132 g) than snakes fed in the laboratory following at least seven days of fasting (mean = 102 g). Thus, although I could not ensure that snakes had an empty stomach when I fed them it the field, the masses of food consumed suggest that snakes fed in the

field were at least as hungry as snakes fed in the laboratory. Given the thermophilic response of rat snakes following feeding in the field, I expected a corresponding habitat shift towards edges because edges provide the best opportunities for thermoregulation in my study area (Chapter Three). Snakes were more likely than expected by chance to be found in edges following feeding, both when fed in an edge and when fed in the forest. Snakes were also less likely to move following feeding when fed in edges than when fed in the forest and they travelled shorter distances following feeding in edges. However, my prediction that snakes should be more likely to move following feeding on thermally challenging days was not supported. Maximum daily temperature or maximum daily radiation were not good predictors of whether or not snakes moved following feeding. One potential explanation for why environmental conditions did not affect whether or not snakes moved following feeding is that I did not have data for very thermally challenging days. In fact, the lowest maximum temperature on a feeding day was 19.4°C, whereas the lowest maximum radiation was 0.54. On very thermally challenging days, black rat snakes used retreats buffered from ambient conditions and were thus not accessible for feeding. Consistent with the thermophilic response I documented for free-ranging black rat snakes following feeding, fed rat snakes were more likely to be found basking than other rat snakes radio-tracked as part of a larger telemetry study. Also, consistent with the need for black rat snakes to reach thermally favourable

sites following feeding, fed rat snakes were more likely to have moved than other rat snakes radio-tracked as part of a larger telemetry study. Overall, it does seem that edges are used primarily for thermoregulation because after a large meal that elicits thermophily (a time when foraging requirements are presumably unimportant), black rat snakes preferentially use edges. If edges were not used for thermoregulation, snakes would likely have been found in forests as often as in edges following feeding.

Table 4-1: Summary of the thermoregulatory behaviour of black rat snakes prior to and after feeding in the laboratory thermal gradient.

Thermoregulation variable	Before feeding	After feeding	Significance
Mean T_b	27.4 ±0.7°C	29.2 ±0.8°C	Paired $t_{(12)} = 2.29$ $p = 0.02$
25% quartile	25.6 ±0.8°C	27.8 ±0.9°C	Paired $t_{(12)} = 2.71$ $p = 0.009$
75% quartile	29.5 ±0.8°C	30.6 ±0.7°C	Paired $t_{(12)} = 1.24$ $p = 0.12$

Table 4-2: Summary of the thermoregulatory behaviour of black rat snakes prior to and after feeding in the field.

Index	Period	Before feeding	After feeding	Significance
Mean T_b *	Whole day	26.5 ±0.5°C	26.7 ±0.5°C	$F_{(1,13)} = 0.013$ $p = 0.91$
	Day only	26.8 ±0.6°C	27.2 ±0.6°C	$F_{(1,13)} = 0.239$ $p = 0.63$
$d_e - d_b$	Whole day	3.3 ±0.7°C	4.4 ±0.6°C	Paired $t_{(7)} = 1.255$ $p = 0.12$
	Day only	3.1 ±0.8°C	4.1 ±0.6°C	Paired $t_{(7)} = 0.891$ $p = 0.20$
Ex	Whole day	32 ±12%	52 ±9%	Paired $t_{(7)} = 0.775$ $p = 0.23$
	Day only	31 ±13%	51 ±8%	Paired $t_{(7)} = 1.220$ $p = 0.13$

*Mean T_b was adjusted for differences in mean T_e by using least-square means.

Table 4-3: Summary of the published literature on the presence or absence of laboratory postprandial thermophily (PPT) in snakes indicating the number of experimental animals involved and the type of food fed to the experimental animals. The methods column indicates whether a thermal preference chamber (TPC) was used and how the body temperatures of snakes were determined.

Reference	Species	N	Food	Methods
PPT present				
Regal 1966	*Coluber constrictor*	1	Mice	TPC + gastric probes
McGinnis 1969	*Boa constrictor*	1	Rats	TPC + gastric transmitters
Lysenko and Gillis 1980	*Thamnophis sirtalis*	4	Fish	TPC + substrate temperatures
Slip and Shine 1988	*Morelia spilota*	15	Mice + rats	TPC + coelomic transmitters
Bozinovic and Rosenmann 1988	*Phylodryas chamissomis*	3	Mice + lizards	TPC + cloacal probes
Gibson et al. 1989	*Thamnophis sirtalis*	5	Fish	Choice of hot + warm shelters
Lutterschmidt and Reinert 1990	*Nerodia sipedon*	5	Fish	TPC + substrate temperatures

Table 4-3: Continued

Touzeau and Sievert, 1993	*Opheodrys aestivus*	7	Crickets	TPC + cloacal probes
Present study	*Elaphe obsoleta*	13	Chipmunks	TPC + coelomic transmitters
PPT absent				
Kitchell 1969	*Heterodon platyrhinos*	1	unknown	TPC + cloacal probes
	Thamnophis sirtalis	3		
	Nerodia sipedon	3		
Lysenko and Gillis, 1980	*Thamnophis sirtalis*	4	Fish	TPC + substrate temperatures
Hammerson 1989	*Masticophis flagellum*	3	Mice + lizards	TPC + gastric transmitters
Tu and Hutchison 1995	*Nerodia rhombifera*	5	Fish	TPC + cloacal probes
Brown and Weatherhead 2000	*Nerodia sipedon*	7	Fish	TPC + coelomic transmitters

Note: The Lysenko and Gillis (1980) study involved two subspecies of *Thamnophis sirtalis*. PPT was present in *Thamnophis sirtalis parietalis*, but absent in *Thamnophis sirtalis sirtalis*.

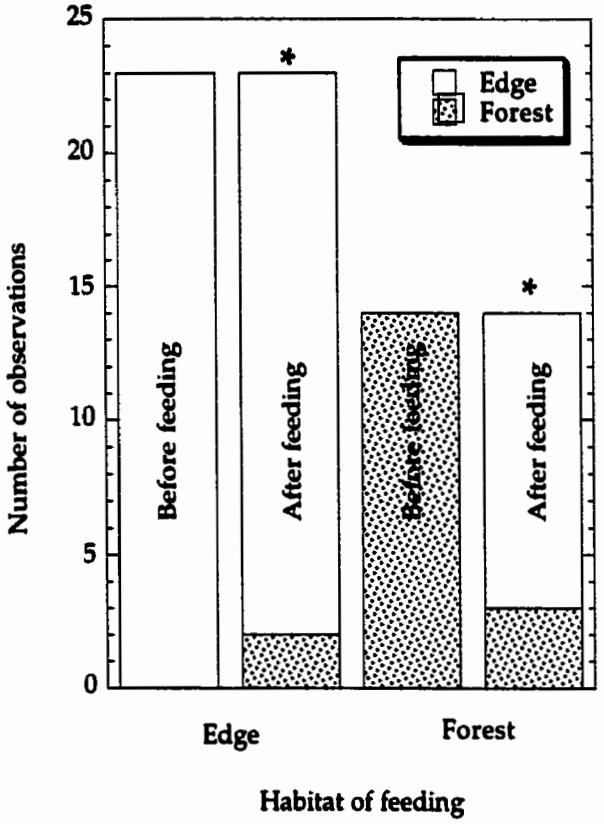

Fig. 4-1: Number of observations of black rat snakes in forest or edges before and after being fed in either edge or forest habitat. Statistically significant differences based on the proportions of the binomial distribution are indicated by an asterisk.

APPENDIX ONE

Anaesthesia and radio-transmitter implantation technique for black rat snakes (*Elaphe obsoleta obsoleta*)

This appendix formed the basis for the following publication:

Blouin-Demers, G., P. J. Weatherhead, , C. M. Shilton, C. E. Parent, and G. P. Brown. 2000. Use of inhalant anesthetics in three snake species. Contemporary Herpetology 2000:4. ISSN 1094-2246.

Introduction

The miniaturisation of radio-transmitters has allowed research on snakes to advance significantly in the past 20 years (Osgood 1970, Fitch and Shirer 1971). Because snakes often crawl through tight crevices, transmitters must be carried internally. The two main implantation techniques that have been used on snakes are force-feeding and surgical implantation. Force-fed transmitters can cause discomfort to the experimental animals and may alter their behaviour (e.g., cause behaviour normally occurring after feeding: Fitch 1987, Lutterschmidt and Reinert 1990). Also, tracking periods are often limited because the snakes expel force-fed transmitters (Fitch and Shirer 1971). Therefore, most snake researchers now prefer to use surgically-implanted transmitters. Surgery requires that snakes be anaesthetised. Here I report the anaesthesia and surgical implantation techniques I used on black rat snakes.

The first anaesthesia procedures (Karlstrom and Cook 1955, Betz 1962) used hypothermia and/or injectable anaesthetics (e.g., ketamine HCl, pentothal sodium, nembutol). These techniques have several disadvantages, including unpredictable depth of anaesthesia and adverse physiological effects (Bonath 1979, Font and Schwartz 1989, Arena and Richardson 1990, Bennett 1991, Frye 1991, Harvey-Clark 1995). Inhalant anaesthetics are superior to injectable agents both because the depth of anaesthesia can be controlled very accurately and tailored to the patient's response (Bonath 1979, Frye 1991) and

because prolonged recovery times associated with injectable agents are avoided (Arena and Richardson 1990, Frye 1991).

Information regarding the use of inhalant anaesthetics in reptiles is scarce (reviewed by Bennett 1991) and often inadequately reported (McDonald 1976). In addition, even closely related species can respond very differently to the various anaesthetic agents (Aird 1986). Thus, one cannot assume that anaesthesia procedures developed for one species can safely be transferred to another species. Veterinary textbooks only give general guidelines regarding reptilian anaesthesia (e.g. Frye 1991), and the few accounts of procedures for specific species are often based on very few animals (Fagella and Raffe 1987). Thus, anyone initiating radio-telemetry on snakes must rely on trial and error to determine the appropriate anaesthesia procedure for the species of interest. This is likely to result in unnecessary mortality and time loss before a satisfactory technique is developed. As a step towards limiting these problems, my goal was to report my experience anaesthetising and surgically implanting radio-transmitters in black rat snakes.

Materials and methods

Inhalant anaesthetics

There are several general considerations when selecting an inhalant anaesthetic. Obviously the effectiveness of a given anaesthetic for the particular snake species on which it will be used is paramount. However,

effectiveness not only includes the ease with which the desired anaesthetic plane is reached, but also the ease with which recovery is achieved and the risk of mortality while a snake is anaesthetised. Cost of the agent (and the equipment necessary to administer it) is also a consideration. I worked with the three more commonly used inhalant agents: isoflurane, halothane, and methoxyflurane.

Based on its physiological effects on the organism, isoflurane is by far the preferred inhalant agent because it is the least depressing to the cardiovascular system and the least toxic to the hepatic and renal systems (Bennett 1991, Harvey-Clark 1995). Also, of the inhalant anaesthetics, isoflurane is the least soluble in blood and tissue, thus producing the fastest anaesthetic depth changes. However, isoflurane also has a very high vapour pressure (33% at 20°C), which can result in very high concentrations of anaesthetic if the "open-drop" method is used (see Delivery with the "open-drop" method below). Isoflurane is also the most expensive agent to use of the three agents I consider here ($60 US for 100 ml).

Halothane has similar volatility to isoflurane, reaching a maximum concentration of 32% at 20°C (Bennett 1991). Halothane is slightly more soluble in blood than isoflurane and also results in quite rapid anaesthetic depth changes. However, halothane is toxic to the hepatic system. Halothane is the cheapest agent to use among the three I consider ($10 US for 100 ml).

Methoxyflurane has the lowest vapour pressure among inhalant

anaesthetics and a concentration over 3% cannot be obtained at 20°C (Bennett 1991). It is therefore the safest inhalant agent to use in the "open-drop" method. Methoxyflurane is very soluble in blood and results in slow anaesthetic depth changes. This agent is very potent and very toxic to the renal system. Methoxyflurane is less expensive to use than isoflurane, but more expensive than halothane ($212 US for 100 ml, but about 90% less methoxyflurane than isoflurane or halothane is needed to obtain the same results).

Delivery with the "open-drop" method

Of the two methods for delivering inhalant anaesthetics that I consider here, the "open-drop" method is by far the simpler, involving only placement of the snake in an airtight vessel containing volatile anaesthetic. I used this method with halothane and methoxyflurane in my initial trials at anaesthetising black rat snakes and followed Bennett's (1991) recommendation of using 5 ml anaesthetic per 2840 ml of volume of the induction chamber. I used a 4000 ml plastic jar with a screw-on lid for my induction chamber. I applied the agent to cotton swabs which were then placed in the closed jar for 10 min. I agitated the jar to maximise evaporation of the liquid anaesthetic. The actual percentage of agent this creates in the jar is unknown, but will certainly vary with the volatility of the anaesthetic and ambient temperature.

I placed a snake in the jar until it lost its righting reflex. Because I could

see the snake in the jar, I could test the righting reflex simply by tipping the jar and observing the snake's response. If the snakes did not respond to physical stimuli after being removed from the jar, I immediately started ventilating them with a syringe fitted with a catheter (see Recovery from anaesthesia below), continuing throughout the surgery or until some muscle tone returned to the tail. Failure to ventilate deeply anaesthetised snakes in this manner increased the risk of mortality since snakes stop breathing under anaesthesia and thus suffocate if artificial ventilation is not performed. If the snake demonstrated some muscle tone during surgery, ventilation was discontinued until the surgery was completed. If the snakes were still responding to physical stimuli when removed from the jar, they reached surgical depth of anaesthesia if left undisturbed for approximately 10 min. This is likely a consequence of the snakes holding their breath, causing them to continue absorbing anaesthetic from their lungs and air sacs. In this case, I started ventilating the snakes when the body wall was being stitched at the completion of surgery (see Surgical implantation below).

One problem with the "open-drop" method is that the amount of anaesthetic the snakes breathe cannot be controlled precisely. As with all reptiles, snakes can hold their breath for long periods of time and thus absorb the anaesthetic agent in a variable manner (Bennett 1991). If this is a problem, a sedative such as acepromazine or ketamine could be used 1 h prior to surgery to reduce stress and perhaps prevent snakes holding their breath,

although I did not use this method in my research. Advantages of the "open-drop" method are that it is inexpensive and does not require much equipment, thus making it useful for field situations.

Delivery with a precision vaporiser

The second method of delivering anaesthetic I used on black rat snakes was a precision vaporiser, which involves delivering an exact concentration of anaesthetic mixed in pure oxygen directly to the snakes' lungs, without relying on the snakes voluntarily inhaling the anaesthetic. I used isoflurane as the anaesthetic with this method as this agent provided the fastest induction and recovery times and produced the least side effects of all the anaesthetic agents available at the time the study took place. A vaporiser allows the plane of anaesthesia to be maintained accurately or changed rapidly (Frye 1991). Breathing pure oxygen mixed with anaesthetic during anaesthesia also aids in counteracting the cardiovascular depression and hypoxia expected during any anaesthesia. Because of a snake's small lung volume, an anaesthetic circuit which minimises re-breathing of expired gases is ideal. I therefore used a Bain circuit with oxygen flow rate of 400 - 1000 ml/kg/min (Fig. A1-1).

The minimum equipment required to deliver an anaesthetic with this method is a precision vaporiser specific for the agent to be used (each vaporiser is calibrated for a given agent and can only be used with this agent), an oxygen cylinder, an oxygen regulator, an oxygen rotameter, a ventilation

bag, and a Bain circuit. The oxygen regulator is connected to the oxygen cylinder and allows one to diminish the oxygen pressure from the cylinder prior to delivery to the anaesthesia circuit. The oxygen at a diminished pressure then enters the rotameter that allows adjustment of the oxygen flow rate. The oxygen then goes through the vaporiser where the desired concentration of anaesthetic is mixed in (the desired concentration is adjusted with a dial on the vaporiser). The mixture is finally delivered to the snake via the Bain circuit and a catheter used as an endotracheal tube (Fig. A1-1).

To induce anaesthesia, the patient was placed in a 4000 ml jar. An average-sized black rat snake filled about half the volume of the jar when coiled inside. The lid was screwed onto the jar and the end of the Bain circuit was attached to a hole that had been made in the lid. I then ran isoflurane using the anaesthetic machine into the jar for 2 min to obtain the desired anaesthetic concentration. I then waited until the snake was sufficiently relaxed to allow endotracheal intubation (this was indicated by a very slow righting reflex). When the snake was relaxed, it was removed from the jar and a catheter was inserted in its glottis. For intubation I used a 10 to 14 gauge catheter fitted with an adapter for the Bain circuit (Table A1-1). It is important that the catheter fits tightly into the glottis to prevent leakage around the tube. Once the catheter was in place, the snake was ventilated with the mixture of anaesthetic and oxygen at a rate of approximately six breaths per minute until muscle tone remained only in the tail, at which point the snake

no longer responded to surgical stimuli.

Because the period of anaesthesia required was usually relatively short (< 20 min), I found that the anaesthetic could be discontinued at the start of surgery (while continuing ventilation with 100% oxygen) and still provide anaesthesia long enough to perform transmitter implantation. If a longer period of anaesthesia was required, the anaesthetic percentage was reduced to a maintenance level of 2 - 2.5% after induction.

Recovery from anaesthesia

When bringing snakes out of anaesthesia following surgery, I found that ventilation used in conjunction with physical stimulation promoted more rapid recovery because clearance of inhalant agents is mainly by exhalation. When using the "open-drop" method, I ventilated snakes manually. To ventilate black rat snakes manually, I used a 60 cc syringe fitted with an approximately 15 cm length of 10 to 14 gauge catheter. The other end of the catheter was placed into the glottis. Again, it is important that the catheter fits tightly into the glottis to prevent leakage of air around the tube. When ventilating the snakes I used approximately six breaths per minute. During ventilation, the snake's ribs should expand to the same extent as when the snake breathes on its own. The inspiration phase of the ventilation should last approximately 4 sec and the expiration phase approximately 6 sec. Inspiration involves filling the syringe with room air which is then delivered to the lungs via the catheter. Expiration involves withdrawing air from the

snake into the syringe, detaching the syringe from the catheter, and expelling the air into the room (the room must be well ventilated to avoid health hazards), and then filling the syringe with fresh air and repeating the process.

Following anaesthesia with a precision vaporiser, continuing ventilation with 100% oxygen removes anaesthetic from the snake's blood via the lungs and hence accelerates recovery. Thus, I ventilated the snakes for 10 min post-surgery with 100% oxygen using the anaesthesia machine. I then switched to manual ventilation and physical stimulation if the snake had not fully recovered yet. Physical stimulation consisted of frequently changing the snakes' body position and gently pinching their skin and tail. I continued ventilation and stimulation until the snakes were breathing unaided, their righting reflex and tongue-flicking had returned, and the snakes had begun to move freely.

Surgical implantation

The surgical procedures I employed were a modification of those outlined by Reinert and Cundall (1982) and lasted 17 min on average (range = 15 - 20 min), from the first incision to the final suture. The transmitters were implanted 2/3 down the snout to vent length (SVL) from the head. It is important to keep sterile surgical conditions. The snake's body was washed 20 cm on each side of the implantation site. I first washed the snake with betadine and then with 99% isopropyl alcohol. I washed and brushed my hands and forearms thoroughly with betadine soap and wore sterile surgical

gloves. A disposable sterile surgical drape was placed over the snake for the surgery.

The surgical tools (Table A1-1) and the transmitter were taken out of the cold sterile solution (Germex®) and rinsed with sterile fluid (0.9% saline or lactated Ringer's solution) poured on them with a 20 cc syringe. To secure the transmitter to a rib (see below), I first attached the suture material to the transmitter by making a knot around the transmitter (Model SI-2T, Holohil Systems Inc., Carp, Ontario, 8.6 g, 20 months battery life at 20°C) using the specially designed groove in the transmitter. The surgical knot is made by wrapping one loop of the thread starting from the inside around the Olsen-Hegar needle drivers with one hand. The needle drivers are then used to grab the other end of the thread. The knot is tightened by pulling both sides of the thread in opposite directions. The knots should be tightened in alternating directions so as to obtain square knots which will not slide. Four throws should be made for each stitch. Enough of a free end of suture material was left to tie a knot around a rib, and the needle was left attached to the suture. I cut a 5 cm hole in the middle of the disposable sterile drape material which is put over the snake during the entire surgery so as to keep the incision and the adjacent work area sterile.

I made a 2 - 3 cm incision lengthways between the second and third rows of scale through the epidermis with the scalpel. If excessive bleeding occurred during the surgery, I squeezed the bleeding vessel with the

Mousquito haemostat or applied pressure for 2 min on the bleeding vessel to stop the bleeding. Then, I cut lengthways through the body wall, the ribs, and the peritoneum with the Tenotomy scissors. The incision should be 2 - 3 ribs wide, which provides the minimum space necessary to get the transmitter into the body cavity. Care must be taken while cutting the body wall so as not to pierce the air sacs. To avoid this problem, I lifted the body wall with the Adson forceps and then made the incision with the scissors.

The transmitter was inserted posteriorly with the antenna towards the snake's head and with the side of the transmitter where the antenna arises facing the exterior. I ensured that none of the organs was excessively compressed and left the antenna outside the coelomic cavity. I stitched the transmitter to the first intact rib with a strong knot. In constricting and narrow-bodied snakes like black rat snakes, the transmitter must be attached to the ribs to prevent it from migrating in the body cavity. Migration would compress organs, increase the risk of skin abrasion, and infection could result. The body wall was then sutured with stitches 3 mm apart, including the peritoneum in the stitch whenever possible to help the healing process.

A hollow 2 mm diameter stainless steel rod was used as a guide to get the antenna running up under the skin. The rod was inserted in a parallel fashion anteriorly from the incision between the epidermis and the body wall. I fed the antenna into the rod, making sure that no kinks remained. The rod was lifted with the Adson forceps at the location of the exit hole and a

small incision was made with the scissors between two scales. I grabbed the end of the rod with the Mousquito haemostat and slid it out carefully. The epidermis was then stitched by including 1 mm of skin on each side of the incision in each suture. I used one suture between each pair of scales located on opposite sides of the incision. Suturing the skin in between scales allowed faster healing and produced a cleaner scar than suturing the scales themselves. Lastly, I stitched the anterior exit hole of the rod. Two coats of spray bandage (Opsite®) were applied over the stitches.

When removing a transmitter, I followed the same steps but I made the incision posterior to the original incision because the scar tissue is weaker than the original tissue.

Post-surgical care

While the snake was waking up, I injected it with sterile fluids (0.9% saline or lactated Ringer's solution at a dosage of 50 - 100 ml/kg) into the coelom to help the healing process and to avoid any damage caused by the antibiotic if the snake was dehydrated. Fluids were given in the lower one fourth of the body anterior to the cloaca with the needle directed at a 45° angle between ventral scales and off the midline. The needle was placed very shallowly to avoid penetrating abdominal viscera. I drew back the needle plunger before injecting to ensure the needle was not in a blood vessel. If a prominent bulge occurred at the site, I used several sites. Then the snake was injected with gentamicin sulfate (reptile dose = 2.5 mg/kg). This antibiotic is

potentially nephrotoxic in a dehydrated animal, which is why I had injected the snakes intra coelomicly with saline solution. Gentamicin sulfate has an excellent activity against gram-negative bacteria, which are the main snake pathogens, and a single subcutaneous injection maintains the therapeutic blood levels of antibiotic for 72 hrs. Because I gave a second injection before release (72 hrs post surgery), gentamicin sulfate gave a total of 144 hrs of antibiotic coverage. This period of antibiotic coverage was important because reptiles heal slowly and implants predispose snakes to infection. The renal portal circulation is situated in the posterior third of the body in snakes. Injecting the antibiotic in this region would cause it to be passed through the kidneys (thus causing elimination) before being circulated in the body. Hence, I made several subcutaneous injections (never more than 0.10 cc at a time) in the anterior part of the body so as to minimise the impact of this antibiotic on the snake's tissue. I always drew back the needle plunger before injecting to ensure the needle was not in a blood vessel.

The snakes were then kept in captivity and monitored for 3 days and provided warmth. Water was not provided because if the snakes soaked themselves, it could have caused the premature melting of the stitches. After 3 days, a second injection of gentamicin sulfate at the same dosage was given and the snakes were released at the point of capture.

Results

Before presenting data on induction times, anaesthetic concentrations and guidelines for ventilation, it is important to stress that these are generalisations and should be tailored to the depth of anaesthesia of individual snakes. For example, a snake under high stress will likely produce substantial amounts of adrenaline. Adrenaline can counteract the effect of the anaesthetic and lead to major variation in dosages required. Some authors suggest injectable pre-anaesthetic sedation with acepromazine or ketamine to achieve more consistent results with inhalant anaesthesia (Bennett 1991). However, use of a pre-anaesthetic may increase recovery time. I found that once I obtained experience with inhalant anaesthesia in a few snakes, I was able to achieve consistent surgical anaesthesia and rapid recoveries with the inhalant agent alone.

"Open-drop" method

I used halothane in the "open-drop" method on seven black rat snakes (510 g - 925 g). Black rat snakes lost their righting reflex and reached surgical depth of anaesthesia after an average of 29.6 min (range = 23 - 33 min) spent in the jar. However, there was substantial individual variation in the response to this agent (this has also been noted by others (Hackenbrock 1963)). I used the amount of halothane suggested by Bennett (1991) on five black rat snakes (7 ml of halothane in a 4000 ml jar). It worked well for one snake, two

snakes did not revive from anaesthesia and died, and two snakes took over 70 min to recover. I used half this amount of halothane on two snakes and both began to revive in the middle of the surgery. Therefore, it seems that the halothane dosage required to keep black rat snakes at a surgical plane of anaesthesia long enough for transmitter implantation is often lethal or requires very long recovery periods. Aird (1986) also reported high mortality when using halothane on *Crotalus*. Halothane appears unsuitable for black rat snake anaesthesia.

I used methoxyflurane in the "open-drop" method and delivered the maximum achievable methoxyflurane concentration by soaking the cotton swab with the agent. The obtained concentration was not enough to bring two black rat snakes (670 g and 840 g) to a surgical depth of anaesthesia. The snakes were still very responsive even after 35 and 37 min, respectively, spent in the jar. Thus, this agent appears not to provide sufficient anaesthesia in black rat snakes, although it has been used successfully on other species such as *Thamnophis* (Charland 1991) or *Crotalus* (Aird 1986).

Precision vaporiser method

I used isoflurane in a precision vaporiser to anaesthetise 61 individual black rat snakes (375 g - 1210 g) at total of 105 times and all recovered. I recorded induction times and recovery times for 26 individuals. I used a 5% isoflurane concentration for induction in the jar which took on average 12.6 min (range = 10 - 17 min). I initially tried inducing the snakes at less than 5%

isoflurane but was unable to achieve surgical anaesthesia. After intubation, I continued running 5% isoflurane and the snakes reached surgical depth of anaesthesia after an average of 18.2 min (range = 12 - 26 min). I used ANCOVA to assess the effect of sex and mass on induction time. The interaction term was not significant ($F_{(1,24)} = 2.02$, $p = 0.16$), and neither sex ($F_{(1,25)} = 0.40$, $p = 0.53$) or mass ($F_{(1,25)} = 0.55$, $p = 0.46$) was significant, indicating that neither sex or mass had an effect on induction time.

Maintenance at a surgical depth of anaesthesia can be achieved by delivering 2.25% isoflurane after induction. Recovery was complete after an average of 12.2 min (range = 1 - 31 min) post surgery. I used ANCOVA to assess the effect of sex and mass on recovery time. The interaction term was significant ($F_{(1,24)} = 7.01$, $p = 0.01$), indicating that mass had a different effect on recovery time for males and females. I thus conducted two separate simple linear regression analyses to assess the effect of mass on recovery time. For females (n = 17), mass had a significant effect ($r = -0.57$, $p = 0.01$) on recovery time. The negative correlation coefficient indicated that heavier females recovered faster from anaesthesia that lighter females. For males (n = 11), mass had no significant effect ($r = 0.28$, $p = 0.39$) on recovery time.

Discussion

There is substantial variation within species in how individuals respond to a given anaesthetic agent. This variation potentially could be a

function of individual differences in stress levels, condition, age, body temperature, size, or other factors. The effects of these factors on the response to anaesthesia have never been investigated and our understanding of this variation is thus very limited. My preliminary results with black rat snakes indicated that sex did not seem to affect anaesthesia induction time. However, larger female black rat snakes recovered faster from anaesthesia than smaller females, although mass had no effect on recovery time for male black rat snakes. This inconsistency of the effect of mass on induction and recovery times in rat snakes suggests that further investigation would be worthwhile to determine whether a real effect exists but is confounded by other factors.

Even if we can not predict how an individual snake will respond to anaesthesia, we can reduce individual variation by delivering the anaesthetic agent in a more consistent manner. Reducing this variation was the main advantage of delivering anaesthetic with a precision vaporiser rather than with the "open-drop" method. The vaporiser permits accurate control of the amount of anaesthetic that the patient breathes. This method also avoids the problems associated with snakes holding their breath found with the "open-drop" technique. The use of a vaporiser also allows the patient to breathe oxygen during surgery, which promotes more rapid recovery. Finally, the use of a vaporiser avoids waste of anaesthetic because all the anaesthetic is delivered to the patient. This becomes a more important consideration when dealing with expensive agents like isoflurane. For all these reasons I advocate

the use of a precision vaporiser over the "open-drop" method.

In addition to variation in how individuals within species respond to anaesthesia, there is substantial variation among species, as indicated by previous studies (e.g., McDonald 1976, Bonath 1979, Aird 1986, Font and Schwartz 1989, Arena and Richardson 1990). For example, the technique used to anaesthetise other snakes (halothane in the open-drop method, Bonath 1979) was inappropriate for use on black rat snakes. This required a trial and error approach to design a technique suitable for black rat snakes, resulting in lost time and two fatalities. At present such a trial and error approach is necessary whenever one attempts to anaesthetise an individual of a species for which no published anaesthesia information is available. Given my results with three different agents, and both physiological and practical considerations, I recommend isoflurane as the first anaesthetic to assess when working with a species for the first time. All the snakes I anaesthetised with isoflurane revived whereas I observed mortality rates which would be considered unacceptable by veterinarian standards when using halothane (2/7 for the black rat snake).

Ideally one would like to be able to predict how different species will respond to different anaesthetics. There is insufficient information in the literature at present to determine whether the response to a given agent follows taxonomic lines, or is related to other natural history characteristic of different species (e.g. size; aquatic vs. terrestrial vs. arboreal species). Because

of the obvious benefits to future researchers of being able to predict which anaesthetic will be best for their species, I encourage people with experience anaesthetising different snake species to communicate this information (both positive and negative) when publishing the results of their research.

Table A1-1: List of equipment, instruments, materials, and supplies used for surgical implantation of radio-transmitters in black rat snakes.

Category	Item
Anesthesia circuit	Induction jar (4000 ml)
	Oxygen cylinder ("E" tank)
	Medical oxygen compressed gas regulator
	Oxygen flowmeter
	Isoflurane vaporizer
	Bain circuit with scavenge ventilation bag (InterMed #BS3353, 1 L)
	Adapter for bain circuit (Rüsch 3 mm)
	Cathether for intubation (14 ga, 3 in)
Instruments	Scalpel
	Tenotomy scissors
	Adson forceps
	Mousquito haemostat
	Olsen-Hegar needle drivers
	Copper tube (2 mm X 250 mm)
Drugs	Isoflurane
	Gentamicin sulfate (5 mg / ml)
Supplies	Carbon steel surgical scalpel blades #10
	Spray dressing (OpSite®, Smith & Nephew #4838)
	Cold sterile solution (Germex® concentrate)
	60 cc syringes with Luer lock
	20 cc syringes with Luer lock
	0.5 cc insulin syringes with 28.5 ga 13 mm needle
	Hypodermic 20 ga 1 in needles with Luer lock
	PDS II 3-0 polydioxanone suture with taper needle (Ethicon #Z316H)
	Surgical Povidone-Iodine 10% scrub brush (E-Z Scrub® #241)
	"Ouchless" non-adherent pads 3in X 4in (Kendall Telfa #2132)
	Isopropyl alcohol 99%
	Betadine solution
	Sterile 0.9% NaCl injection
	Sterile latex gloves
	Sterile incise drape 35cm X 35cm in 60cm X 35cm wrapper (3M #1040)

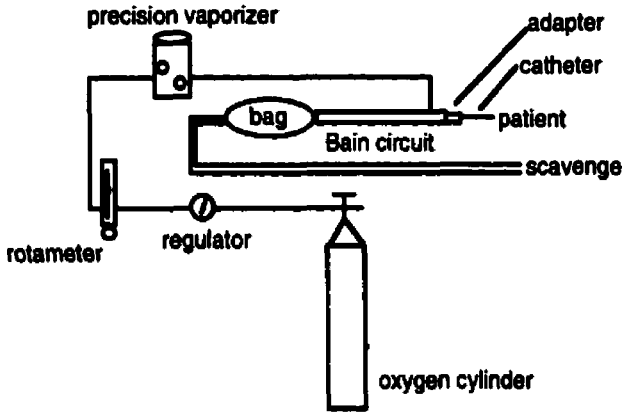

Fig. A1-1: Diagram of the anaesthesia machine circuit used to deliver isoflurane with a precision vaporiser to anaesthetise and implant temperature-sensitive radio-transmitters in Ontario black rat snakes.

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