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Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes

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Abstract We investigated the link between thermal quality and the effectiveness of thermoregulation in milk snakes in a thermally challenging environment. We defined thermoregulatory effectiveness as the extent to which an individual maintains its body temperature (T_b) closer to the preferred range (T_{set}) than allowed by the thermal quality of its environment. We defined thermal quality as the magnitude of the difference between operative environmental temperatures (T_e) and T_{set}. Because ectotherms regulate body temperatures through choice of habitat and behavioural adjustments, we also examined the link between thermoregulation, habitat use and behaviour. During 2003–2004, we located 25 individuals 890 times, and recorded their T_b. Thermal quality was lower in the spring and fall than in the summer, and was lower in forests than in open habitats. Milk snakes thermoregulated more effectively in the spring than in the summer and fall, and more effectively in the forest than in open habitats. Milk snakes had a strong preference for open habitats in all seasons, which was likely to facilitate behavioural thermoregulation. The preference for open habitats was equally strong in all seasons and, therefore, the higher effectiveness of thermoregulation was not a result of altered habitat use. Instead, milk snakes modified their behaviour and were seen basking more and moved less in the spring than in the summer.

Keywords Radio-telemetry · Ectotherm · *Lampropeltis triangulum* · Ontario · Seasonality · Thermoconformity

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Introduction

There are approximately 49,400 species of vertebrates, of which over 70% are ectotherms. As body temperature variation affects most physiological and developmental processes (Peterson et al. 1993) and consequently has a large effect on fitness (Christian and Tracy 1981; Huey and Kingsolver 1989), ectotherms should thermoregulate to maintain body temperatures (T_b; Table 1) that maximize performance and, therefore, fitness (Christian and Tracy 1981; Huey and Kingsolver 1989). The extent of thermoregulation, however, varies among species and among environments. Some species are thermoconformers that are active under a broad range of T_b (Ruibal 1961; Hertz 1992), while others are precise thermoregulators that are active under a narrow range of T_b (Adolph 1990). One factor believed to account for this variation in thermoregulatory strategy is the thermal quality of the environment (Huey 1974; Huey and Slatkin 1976). To understand the ecology and evolution of thermoregulatory behaviour, we need to determine the effect of thermal quality on the extent of thermoregulation.

The thermal quality of the environment directly affects the amount of time and energy that must be expended to maintain T_b within the preferred range. In low thermal quality environments, such as our study area (Fig. 1), environmental temperatures (T_e; Table 1) are far from the optimum for performance, and this makes maintaining optimal T_b costly in time and energy. On the other hand, thermoconformity in low thermal quality habitats entails high fitness disadvantages because thermoconformity then results in T_b that translate to very poor performance. Evidence suggests that ectotherms thermoregulate more effectively when thermal quality is low (Blouin-Demers and Weatherhead 2002; Blouin-Demers and Nadeau 2005). In the investigation reported here, we investigated how thermoregulatory effectiveness varies with thermal quality for eastern milk snakes. We predicted that the disadvantages of thermoconformity should be higher than the costs associated

Table 1 Definitions of the indices and symbols used in the quantification of thermoregulation

Symbol	Definition
T_b	The body temperatures of a representative sample of free-ranging study animals. Typically measured with cloacal probes or with temperature-sensitive radio-telemetry
T_{set}	The preferred body temperature range or set-point range. Typically measured by the bounds of the central 50% of the distribution of body temperatures selected in a laboratory thermal gradient
T_o	Optimal temperature: the body temperature that maximizes organismal performance
T_e	Operative environmental temperatures: the equilibrium body temperatures that non-thermoregulating animals would experience. Typically measured by the temperature of copper representations of the study animals placed randomly in the habitat
d_b	Accuracy of body temperature of Hertz et al. (1993). Measured by the mean of the deviations of field body temperatures from the preferred body temperature range
d_e	Thermal quality of the habitat of Hertz et al. (1993). Measured by the mean of the deviations of operative environmental temperatures from the preferred body temperature range
d_e - d_b	Index of the effectiveness of thermoregulation of Blouin-Demers and Weatherhead (2001a, b)

with thermoregulation and, therefore, that milk snakes should thermoregulate more effectively when thermal quality is low.

In our study area, the challenge faced by milk snakes is generally to increase T_b. In addition, spring and fall temperatures are lower and more variable than summer temperatures. Thus, firstly we tested the prediction that milk snakes should thermoregulate more effectively in the spring and fall than in the summer. We expected forests to have lower thermal quality than open habitats because little solar radiation reaches the ground in forests, thereby reducing the number of basking sites and lowering T_e. Therefore, secondly we tested the prediction that milk snakes should thermoregulate more effectively while in forests than while in open habitats.

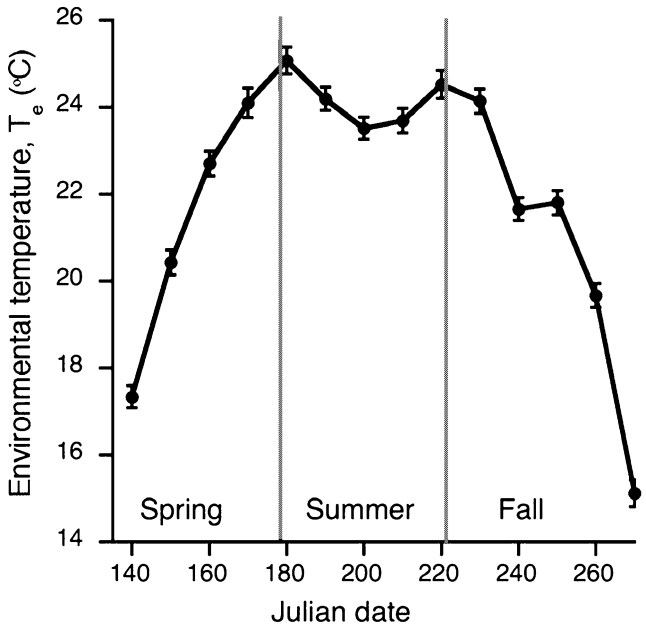

Fig. 1 Mean (±1 SE) operative environmental temperatures (T_e) for all habitats are lower in the spring and fall than in the summer in Ontario, 2003 and 2004

Terrestrial ectotherms are dependent upon obtaining heat from the physical environment and, therefore, reptiles regulate T_b mainly through behaviour and choice of microclimate (Cowles and Bogert 1944; Huey et al. 1989), although this process can be aided by physiological mechanisms (Dzialowski and O'Connor 1999). Thus, thermoregulation is tightly linked to habitat selection and behaviour (Reinert 1993). Empirical data clearly linking the two, however, remain scant. Ectotherms have been shown to modify habitat selection when facing varying temperatures (Christian et al. 1983; Shine and Lambeck 1990; Christian and Bedford 1995; Heard et al. 2004). Nevertheless, few of these studies have demonstrated a clear link between changes in habitat selection and thermal quality of the habitat, and none were conducted in temperate forests where the link between thermoregulation and habitat use should be particularly tight. Thus, thirdly we tested the prediction that milk snakes should alter their habitat use in response to thermal quality and use habitats of higher thermal quality more in the spring and fall when the environment is more thermally challenging.

In addition to predicting that milk snakes should alter habitat selection, we predicted that milk snakes should alter their behaviour in response to variation in thermal quality: they should bask less in summer and in open habitats. Because thermoregulation only requires short movements (shuttling between sun and shade) while most other activities, such as foraging, require more extensive movements, we also predicted that milk snakes should move less in the spring and fall and in open habitats.

Other major factors contributing to habitat selection and behaviour in terrestrial squamates are sex (Shine and Lambeck 1985; Reinert 1993), reproductive condition (Reinert 1984, 1993; Blouin-Demers and Weatherhead 2001a), prey abundance (Reinert 1993; Madsen and Shine 1996) and predator avoidance. We briefly examined the effect of these factors on milk snake habitat selection to ensure that the patterns we observed with thermal quality were real.

Methods

Study area and study species

We conducted this study on milk snakes (*Lampropeltis triangulum*) from August 2002 to November 2004 at the Queen's University Biological Station, 150 km south of Ottawa in eastern Ontario (44°34′N, 76°19′W). The study area was approximately 8×3 km^2 and comprised mainly of second growth deciduous forest with numerous granite rocky outcrops, old fields, small lakes and marshes.

Radio-telemetry

We captured experimental animals opportunistically throughout the active season or at ratsnake hibernacula during spring emergence (Blouin-Demers et al. 2000a). From all captured individuals, we selected a subset of animals that were large enough (at least 130 g) to bear the radio-transmitter (Model SB-2T, battery life of 12 months at 20°C, Holohil Systems, Carp, Ontario, Canada). The transmitter weighted 5.5 g and never exceeded 5% of the snake's mass. We aimed at implanting transmitters in an equal number of females and males, but this was not possible because females are smaller than males and because we captured fewer females than males. We excluded gravid females from our analyses. Transmitters were implanted under sterile conditions and isoflurane anaesthesia (Blouin-Demers et al. 2000b; Weatherhead and Blouin-Demers 2004).

We released the snakes at the point of capture 1 day after implantation and located them every 2–3 days using a telemetry receiver and a directional antenna. Upon location, we recorded the UTM (Universal Transverse Mercator) coordinates with a GPS, the position and the behaviour of the snake. We used the UTM coordinates to calculate distances travelled between locations.

Body temperature

The radio-transmitters we used emitted a pulse rate that was proportional to temperature. Each time we located a snake, we determined its T_b by measuring the time for ten pulses. Snakes were located when they are most active (08:00–18:00), and the timing of locations was consistent throughout all three seasons. Point sampling of T_b during daytime hours has been shown to overestimate mean monthly T_b (Taylor et al. 2004). Seasonal trends in T_b, however, were similar to trends observed using semi-continuous sampling (Taylor et al. 2004).

Thermal preference

When studying thermoregulation, the first step is to determine the preferred T_b range of the organism (T_{set}; Table 1). T_{set} is believed to match the optimal temperature (T_o; Table 1) for performance (Angilletta et al. 2002; Blouin-Demers et al. 2003), and the former is most often determined by measuring the T_b selected by individuals in an environment where there are no thermoregulatory costs (Huey 1991; Hertz et al. 1993). To do this, we measured T_b in a thermal gradient in the laboratory. The chamber was a plywood box 175×75 cm^2 divided into four sections each 45 cm wide. A linear gradient of approximately 10–40°C was achieved by placing a piece of flat sheet metal on top of a cool water coil under one end of the chamber and a piece of sheet metal over heating pads at the other end of the chamber. Constant and homogeneous illumination was provided by a fluorescent light. We placed radio-implanted individuals that did not have an obvious food bulge in the chamber for 24 h to become accustomed to the set- up and then recorded their T_b every 15 min between 06:00 and 22:00 in late July 2004. We used the bounds of the central 50% of observed T_b to determine T_{set} (Hertz et al. 1993; Christian and Weavers 1996; Blouin-Demers and Weatherhead 2001b).

Accuracy of T_b

Following Hertz et al. (1993), we measured the accuracy of T_b (d_b; Table 1) by calculating the mean deviations, in absolute value, of T_b from T_{set}. When T_b was above T_{set}, d_b was calculated as the difference between T_b and the upper bound of T_{set}. When T_b was below T_{set}, d_b was calculated with the lower bound.

Operative environmental temperatures and thermal quality

Operative environmental temperature refers to the T_b available to the ectotherm in the field (Bakken 1992). These temperatures can be determined by placing physical models with similar thermal characteristics as the animal in the various habitats available. Using these models and concurrent environmental conditions, one can build predictive equations that determine the T_b available to the ectotherm in various habitats at all times, thus providing the T_b that a non-thermoregulating (behaving randomly with respect to thermal quality) animal would achieve. Similar to d_b, the thermal quality of the habitat (d_e; Table 1) was measured by calculating the deviations of T_e from T_{set} in absolute value (Hertz et al. 1993).

Milk snake models were made from 1.9-cm diameter copper pipe cut to 30 cm and painted ruddy brown to approximate the reflectance of eastern milk snakes (Peterson et al. 1993). We sealed one end of the model by soldering a copper cap and placed an iButton (±1°C) temperature data logger (Dallas semiconductor, Dallas, Tex.) inside to record T_e. The other end of the model was sealed with a rubber stopper and silicone. We calibrated the models by placing one in the open for two

sunny days beside a fresh road kill milk snake (190 g) with an iButton recording temperature every 10 min in the body cavity.

To record T_e, we placed the models in ten microhabitats. Our objective was to measure the thermal quality of the various microhabitats available to the snakes in both open habitats and the forest. We placed four models in the forest – one on the ground and three in shelters (under leaves, under rocks and under logs) – and six models in open habitats – three in shelters (under grass, under rocks and under logs) and three on the ground (field, rocky outcrop on grass and rocky outcrop on rocks).

The models recorded temperature in all of the microhabitats every 30 min for 5 weeks (2,048 readings). To determine T_e at all times for all microhabitats, we derived multiple regression equations to predict the model temperature from five climatic variables recorded by the weather station at the field station (temperature, radiation, wind speed, soil temperature and rainfall). We used the combination of variables that gave the highest adjusted R^2 value. We then used the predictive equations to calculate T_e for all microhabitats every 30 min for the duration of the active season in both years. We wanted to quantify the thermal quality of the various microhabitats and to calculate a mean T_e for all microhabitats available to the snakes. We grouped all models into one of the four major habitat types: forest, forest in shelter, open habitat, and open habitat in shelter. For each of these four habitats, we averaged the T_e of all the models for each hour. We then calculated a mean T_e for the four habitats for each hour, under the assumption that snakes have equal access to all four habitats. Because the scale of daily movement by milk snakes (mean distance moved per move = 130 m) was larger than the scale at which those habitats occur (mean diameter of habitat patches = 78 m), this seemed to be a reasonable assumption. We used the same method to calculate a mean d_e for each hour.

As snakes were located once every second day, we felt it was more appropriate to use only the T_e's at the time the individual was located as a measure of the thermal environment available to the individual. Therefore, we extracted the mean T_e and the mean d_e for the hour during which each individual was located. Thus, for each snake location we had a T_b and associated T_e and d_e. We used these values to calculate mean T_e and d_e for each snake.

Indices of thermoregulation

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

Using d_b and d_e, we calculated the effectiveness of thermoregulation as the difference between d_e and d_b (Blouin-Demers and Weatherhead 2001b; Table 1). This is an open-ended scale that measures the departure from thermoconformity. Thus, negative numbers represent animals that avoid thermally favourable habitats, zero represents thermoconformity, and positive numbers represent animals that thermoregulate to some degree. The magnitude of the departure from thermoconformity is a measure of thermoregulatory effectiveness.

When calculating the indices to examine thermoregulation differences between seasons, we assumed that all habitats were available to the snakes and we averaged d_e for all habitats. When calculating the indices to determine effectiveness of thermoregulation while in the various habitats, however, we only wanted to know the extent to which the individual was exploiting the thermal opportunities available in that particular habitat. Thus, in those instances we calculated d_e using temperatures available in that habitat only, ignoring temperatures that might have been available in other habitats.

By determining if milk snakes alter their effectiveness of thermoregulation by season or by habitat, we established whether broad trends in thermal quality result in changes in thermoregulation. We also wanted to investigate whether snakes respond to thermal quality on a shorter time scale: i.e. do they respond to daily variation in thermal quality? Therefore, for each individual snake we regressed all values of d_b on their associated values of d_e and examined the distribution of slopes for all snakes. We used this regression to avoid the problem of regressing effectiveness of thermoregulation (d_e - d_b) on d_e where d_e would be present on both axes. It can be shown mathematically that testing for a slope of 1 while regressing d_b on d_e is equivalent to testing for a slope of 0 while regressing d_e - d_b on d_e (Blouin-Demers and Nadeau 2005). In our approach, a slope of >1 indicates that thermoregulatory effectiveness decreases when thermal quality of the environment decreases. A slope of <1, however, demonstrates that thermoregulatory effectiveness increases as thermal quality decreases. We used a *t*-test to determine if the mean of the slopes was significantly different from 1.

Habitat use and behaviour

To measure habitat use, we classified each snake location as being in open habitat (field, rocky outcrop, marsh) or forest. Milk snakes were located once every 2–3 days, which was ample time for an individual to transverse its entire home range. Therefore, if a snake did not move between locations we regarded this as the snake's choice and included it multiple times in the analysis. The classification of locations was done with ARCVIEW 3.2 (ESRI 2000) using a classified 4-m resolution IKONOS image of the study area. We used GRASS 5.0.2 (GRASS Development Team 2003) to georeference the image and to perform a supervised sequential maximum

a posteriori (SMAP) classification. We classified the image into five habitat classes with the "i.smap" command: field, open water, forest, marsh, and rocky outcrop. Any snake location in the forest, but within 10 m of an edge with an open habitat, was classified as being in the open habitat because temperature and radiation have been shown to have significant effects on the microclimate for at least 10 m into forest (Matlack 1993; Murcia 1995). Also, the accuracy of the GPS unit and the accuracy of the satellite image were both ±5 m, combining for a maximum error of ±10 m. The average patch size for forest was 10.2 ha (diameter of 360 m if circular) and 2,819 m^2 (diameter of 60 m if circular) for open habitats and, therefore, the vast majority of habitat patches were larger than 10 m. Without this 10-m buffer, approximately 50% of the edge locations would be placed in the forest due to positional error. We generated a set of random locations for each individual snake to quantify habitat availability. The number of random locations was equal to the number of snake locations for that individual. To obtain the random locations, we used the chronological series of distances travelled by each individual, but we used a randomly determined bearing between each move. This approach ensured that all the random locations were available to that particular snake. Each random location was classified as being in open habitat or forest.

We classified the behaviour of the snake when located as basking/resting if the snake was immobile, travelling if the snake was moving or concealed if we could not see the snake.

Prey density

The diet of eastern milk snakes is comprised mainly of small mammals (Williams 1988), and the scats of adult milk snakes from our study area consisted almost entirely of mammal hair (J. Row, personal observation). Blouin-Demers and Weatherhead (2001a) quantified small mammal abundance in the study area from 1997 to 1999. We removed prey species not consumed by milk snakes (i.e., chipmunks) from their data set and indexed the abundance of small mammal prey. We took the average number of captures per trap for 100 trapping days in each habitat as an index of prey density.

Statistical analyses

Multiple locations and T_b were recorded for each individual and were not independent. Therefore, we averaged across individuals for the period being examined (e.g., season) before conducting statistical tests. Five individuals were tracked for more than 1 year and were considered to be independent between years. We used the number of times located in the period examined as a weighting variable to give more weight to individuals that had been located more often and, thus, for which the averages were more accurate. We repeated all statistical analyses without the weighting variable and, in all but one case, this did not change the conclusion of the test. If all the interactions in ANOVA were not significant, we removed them from the model and we ran the analysis again; in these latter cases we present only the results of the reduced model. We included sex as a control variable in all ANOVAs, and we tested for differences between the sexes before any data pooling. We did not find any differences between the sexes and, therefore, we do not report the results.

We used JMP 3.2 (SAS Institute 2002) for all statistical analyses. We inspected box plots to determine whether assumptions of normality and homogeneity of variance were upheld. When we found violations, the variables were transformed to meet the assumptions. We reported all means ± 1 SE. We accepted significance of tests at $\alpha = 0.05$.

Results

We located and measured the T_b of 25 individuals (17 males and 8 non-gravid females) 890 times over two active seasons (May to October of 2003 and 2004).

Thermal preference

In total, four males and three females were placed in the thermal gradient. For each individual, we calculated the 75 and 25% quartiles of the distribution of selected T_b. Averaged across all individuals, the mean 75 and 25% quartiles were 29.0 ± 0.70 and 31.0 ± 0.50°C, respectively.

Operative environmental temperatures

We plotted the model temperatures versus the temperatures of a 190-g road kill milk snake, and the temperatures were highly correlated ($R^2 = 0.97$) with a slope of 0.99 and an intercept of 0.59. The difference in temperature, however, was significantly different (mean of differences in absolute value = 0.39 ± 0.64°C, paired $t_{601} = 14.9$, $p \leq 0.001$), indicating that the model tended to slightly underestimate snake temperature. The accuracy of the iButtons used to measure temperature was ±1°C and, therefore, the difference between model and snake was less than the measurement error; as a result, this difference was ignored in subsequent analyses.

We derived multiple regression equations to predict model temperatures in microhabitats available to milk snakes using five environmental variables. All equations explained a large portion of the variation (mean $R^2 = 0.88$, range = 0.77–0.94). We used the equations to determine the T_e and d_e for each hour of the day (08:00–18:00) in all microhabitats from May to October in 2003 and in 2004. To divide the active period in three seasons,

we averaged T_e in all habitats every 10 days. We divided the active season into spring, summer and fall by trying to maximize the temperature difference between the seasons and by trying to keep sample sizes similar. As expected, the temperatures in spring (15 May to 27 June) and fall (16 August to 5 October) are similar and much lower than in the summer (Fig. 1).

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

We used mean d_e and percentage of time d_e was equal to 0 (T_e was within T_{set}) in each season as a measure of thermal quality. In both open habitats and forest, mean d_e was lower in the summer (higher quality) than in the spring and fall (Table 2). Sheltered habitats in the open and forests had the highest mean d_e (poorest quality). The percentage of time when $d_e = 0$ was highest in the summer for all habitats. Open habitats without shelter had the highest percentage of $d_e = 0$ of any habitat in all three seasons.

Overall, open habitats had lower mean d_e (higher quality), higher percentages of time with $d_e = 0$ and wider ranges of T_e (Table 2). These trends were consistent across all three seasons. Therefore, we conclude that open habitats had a higher thermal quality than forest in all three seasons. The difference in thermal quality between habitats, however, was lower in the summer than in the spring and fall. Also, thermal quality was lower in the spring and fall than in the summer for both open habitats and forest.

Body temperatures

We used ANOVA to determine if T_b varied with season and with habitat type. None of the interaction terms was significant. T_b varied significantly with season ($R^2 = 0.27$, $F_{2,72} = 22.71$, $p < 0.001$) and type of habitat ($R^2 = 0.05$, $F_{1,72} = 7.70$, $p = 0.007$). Milk snakes had higher T_b in open habitats, and Tukey-Kramer HSD tests revealed that milk snakes had higher T_b in the spring and summer than in the fall (Table 3).

Effectiveness of thermoregulation

We calculated d_e - d_b in each habitat in each season. We used ANOVA to determine if the effectiveness of thermoregulation differed by season and by type of habitat. There was a significant interaction between the type of habitat and season ($R^2 = 0.04$, $F_{2,114} = 3.57$, $p = 0.032$). In the spring, milk snakes had the highest effectiveness of thermoregulation in forest ($d = 1.20$, $t_{38} = 2.43$, $p = 0.020$). In the summer, the effectiveness of thermoregulation decreased in both open habitats and forest, but it decreased less in open habitats resulting in no significant difference between open habitats and forest ($d = 0.41$, $t_{43} = 0.70$, $p = 0.48$). In the fall, thermoregulatory effectiveness decreased, but it decreased more in open habitats, leading again to a significantly higher effectiveness of thermoregulation in forest than in open habitats ($d = 3.14$, $t_{35} = 4.20$, $p < 0.001$; Fig. 2).

To determine if milk snakes had a different effectiveness of thermoregulation in each season overall, we

Table 2 Mean (±1 SE), maximum and minimum operative environmental temperatures (T_e) recorded in the four main habitats available to eastern milk snakes in Ontario in 2003–2004. Mean (±1 SE), maximum, minimum and percentage equal to zero deviations of operative temperatures from the preferred body temperature range (d_e) of eastern milk snakes

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

Table 3 Mean (±1 SE) body temperatures for eastern milk snakes in three seasons and two habitats in Ontario in 2003 and 2004

	Open	Forest
Spring	27.18 ± 0.44	25.13 ± 0.67
Summer	26.78 ± 0.35	25.99 ± 0.73
Fall	23.92 ± 0.45	23.30 ± 0.67

calculated d_e from temperatures that were available in all habitats and used ANOVA. d_e - d_b varied significantly between seasons ($R^2 = 0.35$, $F_{2,69} = 18.93$, $p < 0.001$). Tukey-Kramer HSD tests revealed that milk snakes thermoregulated more effectively in the spring than in the summer, and in the summer than in the fall.

We regressed values of d_b on their corresponding values of d_e for each individual to determine if thermal quality influenced thermoregulatory effectiveness on a daily basis. The mean slope was 0.69 ± 0.09 and was significantly less than 1 ($d = 1.05$, $t_{29} = 3.39$, $p = 0.001$), indicating that thermoregulatory effectiveness decreases as thermal quality increases (Fig. 3).

Habitat use and behaviour

We calculated the percentage of open habitat for each snake in each season as well as for each random snake in each season. We used an ANOVA to determine if milk snakes used open habitats proportionally more in any of the three seasons. Before running an ANOVA we transformed the percentages of open habitat to ranks to avoid violating the assumptions of ANOVA. Contrary to our prediction, milk snakes used open habitats to the same extent in all three seasons ($R^2 = 0.009$, $F_{2,72} = 0.35$, $p = 0.71$; Fig. 4). We then pooled over season and determined that snake locations were more often in the open than at random locations (Wilcoxon signed rank test $W_{72} = 637.50$, $p < 0.001$).

We analysed the behaviour of the snakes to determine if it varied across seasons. Because these values are categorical, it was impossible to average across individuals before conducting the tests and, consequently, some pseudo-replication resulted. Milk snake behaviour varied significantly across seasons ($\chi^2_4 = 81.95$, $p < 0.001$; Fig. 5): milk snakes were basking/resting most in the spring ($\chi^2_2 = 62.92$, $p < 0.001$), were travelling most in the summer ($\chi^2_2 = 13.05$, $p = 0.001$) and were concealed most in the summer and fall ($\chi^2_2 = 26.30$, $p < 0.001$; Fig. 5). Milk snakes thermoregulated more effectively in the spring than in the summer and fall. They did not, however, alter their habitat use to achieve higher T_bs; instead they basked more.

We predicted that milk snake behaviour should vary by habitat because habitats vary in thermal quality. Contrary to our prediction, however, behaviour was the same in open habitats and forest ($\chi^2_2 = 2.77$, $p = 0.25$). Milk snakes thermoregulated more effectively in forests (lower thermal quality) than in open habitats, but this was not reflected by a significant change in behaviour.

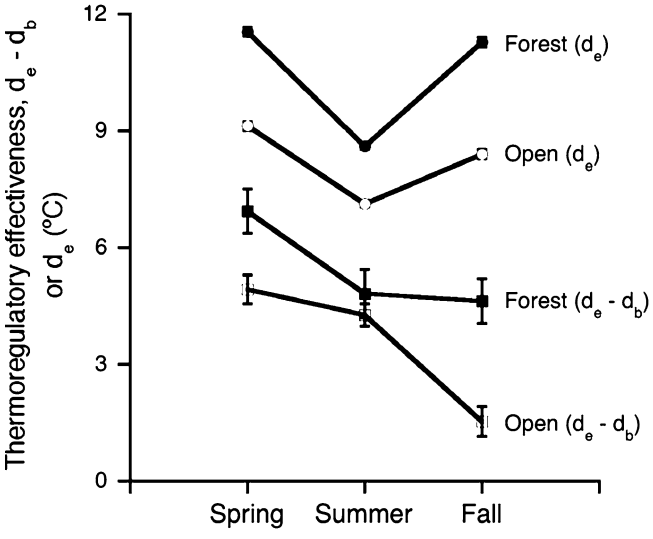

Fig. 2 Deviations (±1 SE) of operative environmental temperatures from the preferred body temperature range (T_{set}) in milk snakes ($n = 25$) in forest (*solid circles*) and open habitats (*open circles*) during the spring, summer and fall. Thermoregulatory effectiveness (mean d_e - d_b ± 1 SE) of milk snakes in forest (*solid squares*) and in open habitats (*open squares*) during three seasons (least square mean weighted by sample size and corrected for sex)

Movements

The distance travelled was log-transformed to meet the assumptions of ANOVA. There were seasonal differences in movements ($R^2 = 0.15$, $F_{1,72} = 6.06$, $p = 0.004$). Tukey-Kramer HSD tests revealed that milk snakes moved farther in the summer than in the spring and fall (Fig. 6).

Prey density

We used ANOVA to determine if prey density varied with habitat type or season. We log transformed the data in an effort to meet the assumptions of ANOVA, but normality could not be achieved. ANOVA, however, is robust to departures from normality, and none of the p values was close to 0.05. There was a significant interaction between season and habitat ($R^2 = 0.15$, $F_{2,147} = 8.09$, $p < 0.001$). Prey density was higher in open habitats in the spring ($Z_{41} = 2.02$, $p = 0.040$), but higher in the forest in the summer ($Z_{80} = 3.00$, $p = 0.003$) and fall ($Z_{21} = 2.83$, $p = 0.005$). If prey density was affecting habitat use, then milk snakes should have been in open habitats more in the spring and in forest more in the summer and fall. This was not the case, however, and milk snakes had a consistent preference for open habitats in all three seasons.

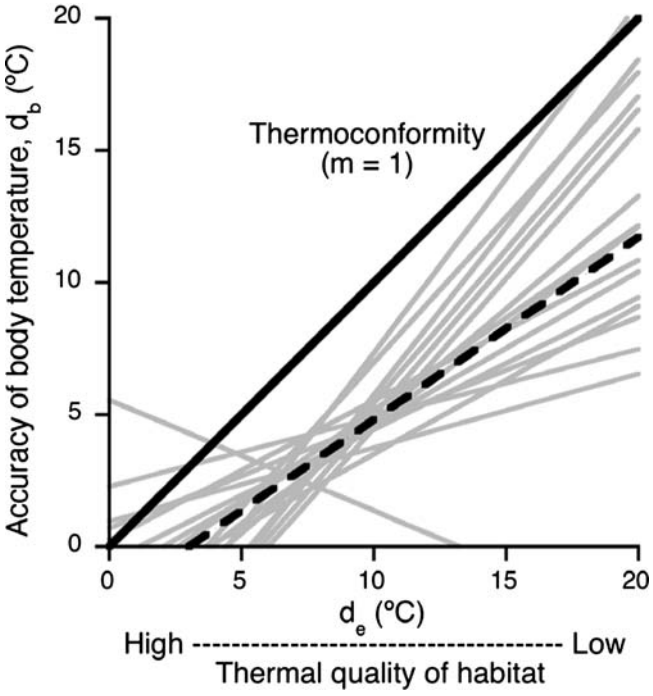

Fig. 3 Milk snakes ($n = 25$) thermoregulate more effectively (high d_e - d_b, see text for details) when the thermal quality of the habitat is low (high d_e, see text for details) during the day (08:00–16:00) in Ontario, 2003 and 2004. This is indicated by the mean slope (*dashed line*) of all individuals (*grey lines*) being significantly less than 1 (*solid line* indicating thermoconformity) ($m = 0.69 \pm 0.09$, $d = 1.05$, $t_{29} = 3.39$, $p = 0.001$)

Discussion

Effectiveness of thermoregulation and thermal quality

The effectiveness of thermoregulation varies with thermal quality, and recent evidence suggests that ectotherms thermoregulate more effectively when the thermal quality is low (Blouin-Demers and Weatherhead 2001b; Blouin-Demers and Nadeau 2005). In our study area, thermal quality was lower in the spring and fall. Consistent with recent evidence and our predictions, milk snakes thermoregulated more effectively in the spring than in the summer. In the fall, however, milk snakes were significantly less effective thermoregulators than in spring and summer. The thermal qualities of all habitats were similar in the spring and fall and, thus, we expected that snakes should adopt similar thermoregulatory strategies. This discrepancy, however, could be attributed to ecological factors other than temperature. During hibernation, reptiles have a temperature-induced torpor in which their metabolic activity is significantly reduced (Gregory 1982). Because of this reduced metabolism, reptiles cannot digest food during hibernation and, therefore, must fast prior to hibernation (Gregory 1982). During this fasting period, milk snakes do not need to elevate T_b to digest, forage or find mates and, therefore, should not engage in thermoregulation because it is costly in energy and in risk of predation. As milk snakes went into hibernation in late September or early October in our study area, the pre-hibernation fasting period could have encompassed the majority of the fall, thereby leading to the reduced effectiveness of thermoregulation.

In the spring and fall, milk snakes had a higher effectiveness of thermoregulation in forest than in open habitats. Because thermal quality was lower in forests, this further supported our prediction that milk snakes should thermoregulate more effectively when the thermal quality is low. In the summer, effectiveness of thermoregulation by milk snakes was the same in open habitats and forest, even though open habitats had a higher thermal quality. Thermal quality in the summer was higher in both habitats, and the difference in thermal quality between open habitats and forest was less in the summer than in the spring and fall. This could explain the similar thermoregulatory effectiveness in both habitats in the summer. On a finer time scale, milk snakes also responded to daily variation in thermal quality and thermoregulated more effectively when thermal quality was low.

Overall, our results reinforce the tight link between thermal quality and thermoregulatory effectiveness. When thermal quality is low, there are high costs in terms of investment in energy, time and exposure to predators that an individual must incur to maintain T_b within T_{set}. In thermally challenging climates (e.g. temperate forests, hot deserts), however, there are also disadvantages to thermoconformity because it will result in extreme T_b's that translate into reduced organismal

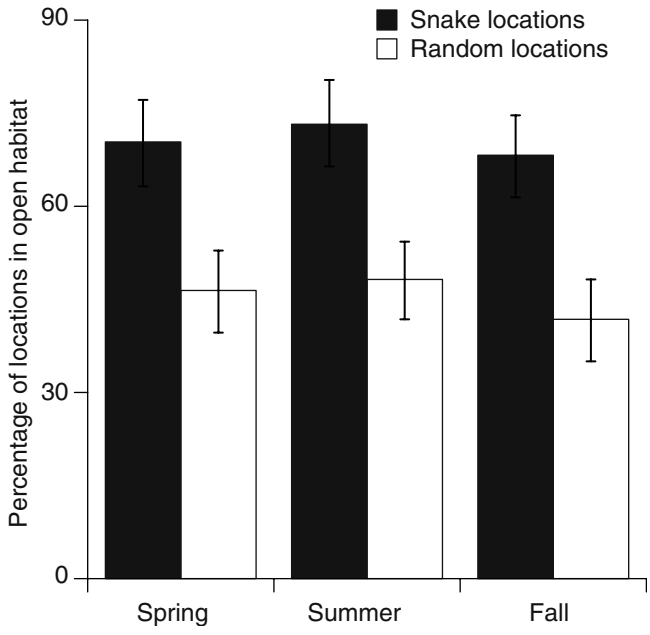

Fig. 4 Mean percentage (±1 SE) of locations in open habitat is consistently higher for milk snake locations ($n = 865$ for 25 individuals, *solid bars*) than random locations (*open bars*) across all three seasons in Ontario, 2003 and 2004

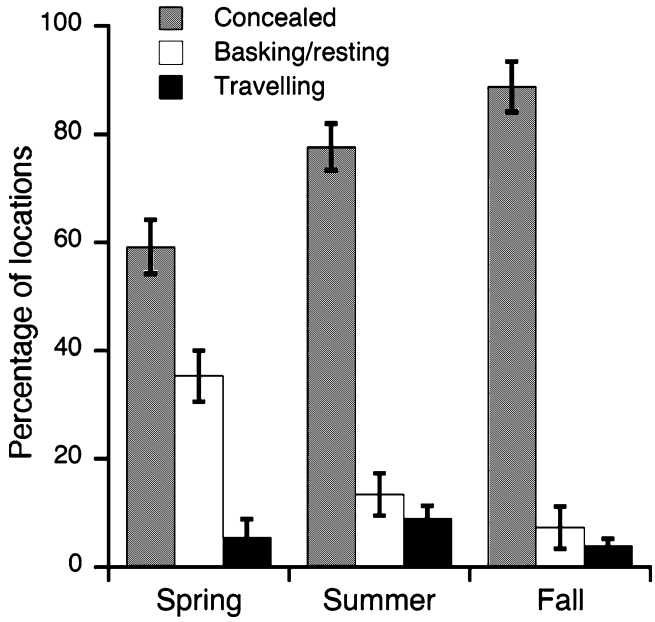

Fig. 5 Percentage (±1 SE) of locations ($n = 865$) where milk snakes ($n = 25$) were concealed (*hatched bars*), basking or resting (*open bars*) and travelling (*solid bars*) during the spring, summer and fall in Ontario, 2003 and 2004

performance. The habitat in Ontario is thermally challenging and, in milk snakes, it appears that the disadvantages associated with thermoconformity when thermal quality is low are more important in influencing the effectiveness of thermoregulation than the costs incurred while thermoregulating.

Habitat use and behaviour

We caught the main prey of adult milk snakes more often in forests than in open habitats in all seasons except the spring. Thus, if milk snakes were selecting habitats based primarily on mammalian prey abundance, they should have preferred forests during the summer and fall. Milk snakes, however, had the same preference for open habitats in all three seasons. Therefore, milk snakes were apparently not preferentially using open habitats for foraging.

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

Ectotherms must obtain heat from their environment and, therefore, they alter their T_b through habitat selection and behaviour. In all three seasons, open habitats provided more T_e within T_{set}. Also, open habitats facilitate thermoregulation by offering a wider range of T_e and by providing the opportunity to shuttle between basking and shelter, which should allow the snakes to maintain T_b within T_{set} for an average of 7 h per day. Maintaining T_b within T_{set} was never possible in forest on average. These observations suggest that thermoregulation is driving the preference for open habitats and, thus, support our prediction that habitat use and thermoregulation should be tightly linked (Christian et al. 1983). This link should be especially tight in thermally challenging environments, such as our study area, where the disadvantages of thermoconformity are much higher than in thermally benign environments.

Because of the link between habitat selection and thermoregulation, we expected milk snakes to select higher thermal quality habitats in the spring and fall when thermal quality was low. Contrary to our prediction, milk snakes did not alter their preference for open habitats through their activity season. Our classification of habitats was coarse and, although we are confident in the results, we realize that habitat selection can occur on much finer scales. Habitat features such as size, orientation and shape of rock have been shown to influence the selection of retreat sites by snakes (Huey et al. 1989; Webb and Shine 1998). Habitat data collected on a finer scale might provide better insight and demonstrate a stronger link between seasonal habitat selection and thermoregulation.

Milk snakes shifted their behaviour seasonally to increase T_b. Instead of using open habitats more often, milk snakes achieved higher T_b in spring by spending more time basking and less time travelling or concealed. By basking more in spring, milk snakes were able to maintain similar T_b in the spring and summer, despite thermal quality being lower in spring. In the fall, milk

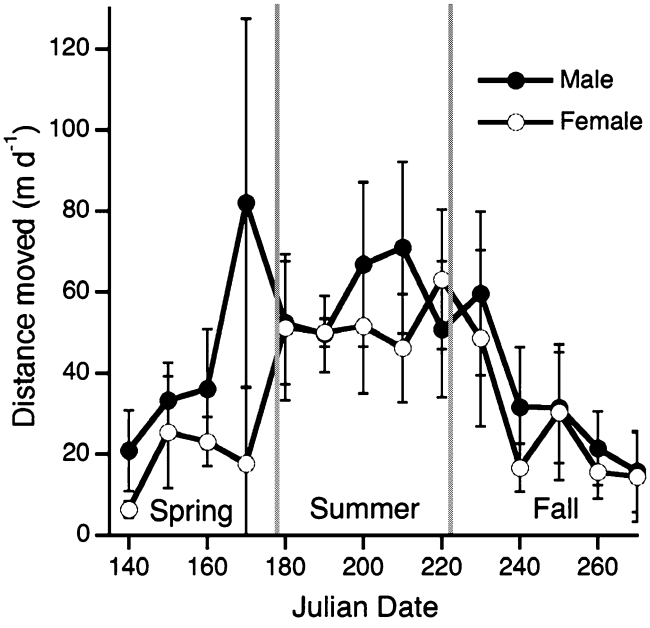

Fig. 6 Distance moved (m/day, ±1 SE) by male ($n = 17$, *solid circles*) and female ($n = 8$, *open circles*) milk snakes in the spring, summer and fall in Ontario, 2003 and 2004

snakes had significantly lower T_b than in the spring and summer, despite the possibility of obtaining T_b similar to the spring. Basking behaviour was at its lowest in the fall and, as a result, T_b and effectiveness of thermoregulation was lower in the fall than in the other two seasons.

Milk snakes moved more in the summer than in the spring and fall. This reflects partly the higher thermal quality and the reduced time investment in thermoregulation in the summer. In the spring, milk snakes had to invest more time into thermoregulation to maintain T_b within T_{set}. Basking or shuttling between sun and shade does not require long movements. In the summer, milk snakes were able to invest less time in maintaining T_b within T_{set} and, therefore, were less constrained by thermal quality in all habitats. This possibly allowed milk snakes to forage more, which requires longer movements. Milk snakes mate from late May to early June (J. Row, personal observation) and, thus, the increased movement rates in the summer could not be related to mate searching. As no gravid females were included in the analyses, the increased movement rates in the summer are not a consequence of nest searching. In the fall, however, milk snakes had the lowest effectiveness of thermoregulation, but still had lower movement rates than in the summer. Again, this is likely to be related to the fasting period before hibernation (Gregory 1982) when milk snakes would not be foraging and, therefore, should not perform risky and energetically demanding behaviours such as travelling.

Conclusion

To gain insight into the ecology and evolution of thermoregulatory behaviour, it is important to determine which factors affect the extent of thermoregulation and which behaviours and mechanisms are used to vary the effectiveness of thermoregulation. Our study demonstrated the influence of thermal quality on the effectiveness of thermoregulation, habitat use and behaviour in an environment of low thermal quality. Milk snakes had a strong preference for open habitats, presumably to facilitate behavioural thermoregulation. Habitat use, however, was unaffected by variation in thermoregulatory effectiveness and, instead, milk snakes basked proportionately more to increase thermoregulatory effectiveness when thermal quality was low. It would be interesting, however, to determine if these trends exist in environments of higher thermal quality, where the disadvantages of thermoconformity are lower. Perhaps this would diminish the importance of thermal quality and attenuate its link with thermoregulatory effectiveness, habitat use and behaviour.

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