Thermoregulation as a function of thermal quality in a northern population of painted turtles, *Chrysemys picta*

A.L. Edwards and G. Blouin-Demers

Abstract: Body temperature affects nearly all processes of ectotherms. Reptiles do not generate sufficient body heat to regulate their body temperature internally and therefore use behavioural thermoregulation. We determined whether thermoregulatory effort varied among seasons in an environment where large temporal differences in environmental temperatures (T_e) exist. We took 31 297 internal body temperature (T_b) measurements from 18 painted turtles (*Chrysemys picta* (Schneider, 1783)) throughout their active season. We estimated T_e with physical models and water temperatures. We measured the range of preferred body temperatures (T_{set}) in a thermal gradient. T_{set} was 21.3–25.0 °C. We used T_b , T_e , and T_{set} to calculate standard thermoregulators, despite inhabiting a high-cost environment. Effort to regulate T_b increased as the thermal quality of the habitat decreased. Thermoregulatory effort was higher when T_{set} could not be achieved. Painted turtles put more effort in thermoregulation in the early season than in the rest of the season. This within-species pattern follows the pattern seen among species. This study is the first to measure T_b internally and to apply standard thermoregulation indices to free-ranging turtles.

Résumé : La température du corps affecte presque tous les processus chez les ectothermes. Les reptiles ne produisent pas assez de chaleur métabolique pour contrôler de l'intérieur leur température corporelle et doivent donc utiliser la thermorégulation comportementale. Nous avons déterminé la variation saisonnière de l'effort de thermorégulation dans un milieu caractérisé par d'importantes fluctuations temporelles de la température externe (T_e). Nous avons obtenu 31 297 mesures de la température interne du corps (T_b) chez 18 tortues peintes (*Chrysemys picta* (Schneider, 1783)) tout au cours de la saison d'activité. Nous avons estimé T_e à l'aide de modèles physiques et de mesures de la température de l'eau. Nous avons déterminé l'étendue des températures corporelles préférées (T_{set}) dans un gradient thermique; T_{set} s'étendait de 21,3–25,0 °C. Nous avons utilisé T_b , T_e et T_{set} pour le calcul des indices standard de thermorégulation (E_x et $d_e - d_b$). Les valeurs des indices E_x (40,7 %) et $d_e - d_b$ (2,4 °C) indiquent que la tortue peinte est un thermorégulateur modéré, bien qu'il habite un environnement à coût élevé. L'effort pour contrôler T_b augmente à mesure que la qualité thermique de l'habitat diminue. L'effort de thermorégulation est plus important lorsque T_{set} ne peut être atteint. Tôt dans la saison, les tortues peintes mettent plus d'effort dans la thermorégulation que durant le reste de la saison. Ce patron intraspécifique est conforme au patron observé entre les espèces. Il est à noter que notre étude est la première à mesurer T_b de l'intérieur et à utiliser les indices standard de thermorégulation chez des tortues libres en nature.

[Traduit par la Rédaction]

Introduction

Body temperature (T_b) affects nearly all ecological, behavioural, and physiological processes of ectotherms (Huey 1982), including heart rate (Spray and May 1972), growth rate (Frazer et al. 1993), locomotion speed (Claussen et al. 2002), prey capture (Wintzer and Motta 2004), and response to predators (Weetman et al. 1998). Because reptiles cannot generate sufficient body heat to maintain a constant T_b , T_b is regulated behaviourally (Cowles and Bogert 1944). Thus, there is a clear link between behavioural thermoregulation and fitness in ectotherms. The extent to which reptiles regu-

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late T_b , however, varies greatly (Christian and Bedford 1995; Diaz 1997; Schauble and Grigg 1998). Thermoregulatory strategies range from thermoconformity (Ruibal 1961), where the organism does not thermoregulate and T_b matches the operative environmental temperatures (T_e), to perfect thermoregulation, where behaviour is used to adjust T_b within a narrow range. Our general goal was to determine how investment in thermoregulation by painted turtles (*Chrysemys picta* (Schneider, 1783)) varies as the thermal quality of the habitat, and thus the cost of thermoregulation, varies.

There are both costs and benefits associated with thermoregulation (Huey and Slatkin 1976). In ectotherms, performance curves are generally an asymmetrical inverted U: performance decreases more precipitously when T_b is higher than the optimum (e.g., Blouin-Demers et al. 2003). Performance is enhanced when T_b approaches a species and process-specific optimum temperature (T_o), which is often close to the species' preferred body temperature (T_{set}) (Hertz 1992; Angilletta 2001). Temperatures below or above T_o re-

Received 15 November 2006. Accepted 13 April 2007. Published on the NRC Research Press Web site at cjz.nrc.ca on 12 May 2007.

sult in a decrease in performance for activities such as feeding (Van Damme et al. 1991), sprinting speed (Claussen et al. 2002), and food assimilation (Zhang and Ji 2004). Thus, the main benefit of thermoregulation is increased performance because thermoregulation allows $T_{\rm b}$ to be maintained close to $T_{\rm o}$. The main cost of thermoregulation is related to energy and time expended in seeking thermoregulation opportunities. When the thermal quality of the environment is low ($T_{\rm e}$ is far from $T_{\rm set}$), more energy and time are required to attain $T_{\rm set}$. Consequently, there are more missed opportunities in low-quality habitats and as a result the cost increases.

In thermally benign environments, the lack of thermoregulation has little consequence because, by definition, T_e is near T_0 . Thus, even without thermoregulation, T_b is close to T_{0} (Shine and Madsen 1996). In thermally challenging environments, like northern climates, the cost of thermoregulation increases, but the disadvantage of thermoconformity also increases because the lack of thermoregulation results in $T_{\rm b}$ being far from $T_{\rm o}$ (Blouin-Demers and Weatherhead 2001; Blouin-Demers and Nadeau 2005). In thermally challenging environments, the disadvantage of thermoconformity may be greater than the cost of thermoregulation and, consequently, more effort is invested in thermoregulation despite the high cost (Blouin-Demers and Weatherhead 2001). Blouin-Demers and Nadeau (2005) demonstrated that lizards living in thermally challenging environments put more effort in thermoregulation than lizards living in thermally benign environments. The pattern was detected among species and our goal here was to determine if the same pattern exists within a species exposed to different $T_{\rm e}$.

The painted turtle ranges across Canada and the continental United States, reaching as far north as 51°N latitude (Cagle 1954). Because our study area in eastern Ontario is within the north-temperate zone, turtles experience broad seasonal variations in temperature that should influence the extent to which they thermoregulate. We wanted to test the prediction that painted turtles put more effort in thermoregulation when the thermal quality of the environment is low, as documented in northern snakes (Blouin-Demers and Weatherhead 2001; Row and Blouin-Demers 2006). Thus, we predicted greater thermoregulatory effort early and late in the active season, when temperatures are cool, and less thermoregulatory effort in the middle of the active season when temperatures are warmer.

By determining if painted turtles alter their thermoregulatory effort by season, we established whether broad trends in thermal quality result in changes in thermoregulatory effort. We wanted to investigate also whether turtles respond to thermal quality on a shorter time scale; do they respond to daily variation in thermal quality. Thus, we tested the prediction that turtles should invest more in thermoregulation on days when the thermal quality of the habitat is low.

Changes in thermoregulatory effort should be reflected in a change in behaviour. Because thermoregulation is accomplished largely through aerial basking in painted turtles, we predicted that turtles should bask more frequently in early and late season than in the middle of the active season. Increased basking should also lead to a concomitant decrease in movements: turtles should move less frequently and shorter distances during intense basking periods.

Previous studies have characterized thermoregulatory effort of turtles through basking length and frequency (Lefevre and Brooks 1995; Krawchuk and Brooks 1998) or carapace temperature (Grayson and Dorcas 2004). Measuring carapace temperature with small data loggers (Nussear et al. 2002) is a considerable improvement over strictly observational studies, but carapace temperature is weakly correlated with internal $T_{\rm b}$, especially during or immediately after basking bouts (see the Results). Because virtually all physiological processes happen within the body, not at the surface of the carapace, the metric under selection for regulation is internal $T_{\rm b}$, not carapace temperature. Although there have been numerous studies on the thermal ecology of turtles (Zimmerman et al. 1994; Lefevre and Brooks 1995; Krawchuk and Brooks 1998; do Amaral et al. 2002; Grayson and Dorcas 2004), this study is the first to measure $T_{\rm b}$ internally in free-ranging turtles and to apply standard thermoregulation indices (Hertz et al. 1993; Christian and Weavers 1996; Blouin-Demers and Weatherhead 2001).

Materials and methods

Study area and study species

We conducted this study in 2004 at the Queen's University Biological Station, 100 km south of Ottawa, Ontario. The study site was a marshy bay in Lake Opinicon. We studied male painted turtles only because females may have higher energetic demands during egg development and, as such, may bask more frequently (Schwarzkopf and Brooks 1985; Krawchuk and Brooks 1998), which would obscure the relationship with the thermal quality of the environment. We captured painted turtles in basking traps and by dip net from a canoe. All of our procedures were approved by the Animal Care Committee at the University of Ottawa (protocol BL-179). Animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1984, 1993).

Body temperature

We selected 26 males (carapace length 136.7 ± 0.96 mm (mean \pm 1 SE), mass 301 \pm 5.8 g (mean \pm 1 SE)) for attachment of a radio transmitter (model SI-2FT, 12 g, 24 months battery life at 20 °C; Holohil Systems, Carp, Ontario) and for surgical implantation of a temperature data logger (Thermochron iButton DS1921, 3.3 g after coating (see below); Dallas Semiconductor, Sunnyvale, California). All males selected for telemetry weighed at least 280 g so that the combined mass in air of the transmitter and the logger never exceeded 5% of the mass of the animal. We secured the temperature-sensitive radio transmitters to the posterior marginal scutes using stainless-steel bolts and locking washers. We then coated the transmitter and bolts with marine-grade silicone to prevent potential snagging in macrophytes. To obtain radio-transmitter temperature, we measured the pulse rate of the transmitter by timing the duration of 10 pulses with a wristwatch. We converted the mean pulse rate to a temperature with a polynomial equation of the calibration curve for each transmitter.

We programmed the data loggers to take readings every 100 min for 142 days from early May until early October. We coated the loggers with Plasti $Dip^{\text{(B)}}$ (Performix, Blaine,

Minnesota) before implantation. The loggers were implanted in the peritoneal cavity, anterior to the hind leg using sterile surgical techniques. Anaesthesia was induced with ketamine and maintained with isoflurane. The individuals were given antibiotics (Enrofloxin[®]) to help prevent possible infection. Turtles were kept for 48 h after implantation and then released at their point of capture.

Both the implanted temperature data logger and the attached temperature-sensitive radio transmitter were necessary for each individual because of the discrepancy between internal T_b and carapace temperature, especially during basking (see the Results). To determine the magnitude of this discrepancy, we placed a fresh turtle carcass in direct sunlight for six 90 min periods. Every 5 min, we recorded temperature 5 cm into the cloaca (in the same general location as the data loggers implanted in experimental animals) and carapace temperature (in the same general location as the radio transmitters) with thermal probes.

We located the study animals two to four times per week throughout the active season. Upon locating an individual, we noted its behaviour (basking or swimming), position, and marked its location on a Universal Transverse Mercator (UTM) grid. From the UTM coordinates, we calculated straight-line distances between locations as an estimate of distance moved.

Operative environmental temperatures

We determined T_e from copper models that were made to imitate the average size and shape of a male painted turtle. We painted the models flat black to match closely the reflectance of painted turtles (Shine and Kearney 2001). We calibrated the models by placing a turtle carcass beside a model and recording the internal temperature of both every 5 min for 170 h. We placed the copper models for several weeks at basking logs and rocks in the sun and in the shade. We used climatic data (air temperature, solar radiation, and wind speed) from the weather station at the field station (located 4 km from the study site) to build predictive equations of model temperature in each microhabitat. From these equations, we generated predicted model temperatures (i.e., T_e) every 10 min at each microhabitat for the duration of the active season.

We placed temperature data loggers at three locations in the study site to measure water temperatures at the surface, 1 m underwater, and 10 cm in the mud. Because of the high temperature conductivity of water, we assumed that T_e in water was the same as water temperature.

Preferred body temperature

We determined T_{set} of painted turtles in a dry thermal gradient because painted turtles bask in air. The thermal gradient was a plywood box (250 cm × 60 cm × 60 cm) divided into two lanes. Coils of plastic tubing circulated cold water at one end of the box, while heating pads were placed at the other end. We used copper sheeting under the box to produce a linear gradient from 10 to 40 °C. We placed the turtles individually in the thermal gradient for 3 days, including a 12 h acclimation period. The turtles were subjected to natural light cycles, but their T_{set} was the same during night and day. Because the lanes of the gradient were narrow (30 cm) and because the ends of the gradient provided temperatures outside the comfort range of turtles, painted turtles tended not to seek cover in the corners of the gradient. We determined T_{set} for each individual from the bounds of the central 50% of the distribution of T_{b} selected in the gradient: the 25th and 75th quartiles (Hertz et al. 1993). We measured T_{set} throughout the active season to account for possible seasonal acclimation.

Quantification of thermoregulation

Hertz et al. (1993) proposed a measure of thermal quality, $d_{\rm e}$, which is the deviation of $T_{\rm e}$ from $T_{\rm set}$ in absolute value. To determine the accuracy of $T_{\rm b}$, Hertz et al. (1993) proposed $d_{\rm b}$, which is the deviation of $T_{\rm b}$ from $T_{\rm set}$ in absolute value. Blouin-Demers and Weatherhead (2001) suggested that $d_{\rm e} - d_{\rm b}$ can be used as an index of investment in thermoregulation where thermoconformity has a value of zero, positive values represent thermoregulatory effort, and negative values represent avoidance of thermally favourable locations. The magnitude of the departure from zero represents the effectiveness of thermoregulation: how much closer is the $T_{\rm b}$ of the animal to $T_{\rm set}$ than is the randomly available $T_{\rm e}$.

To examine how turtles exploited available opportunities for thermoregulation, we also used the index of thermal exploitation (E_x) of Christian and Weavers (1996). E_x is the percentage of time when T_b is within T_{set} for time periods when T_e indicates that T_b within T_{set} can be achieved. We considered T_e within T_{set} to be available for a given time period if T_e was above the lower bound of T_{set} in at least one of the microhabitats because T_e below T_{set} was always available in the coolest microhabitat (see the Results).

To test whether turtles responded to daily variations in thermal quality, we regressed all values of d_b on their associated values of d_e for each individual turtle and then examined the distribution of slopes for all turtles. 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 <1 demonstrates that thermoregulatory effectiveness increases as thermal quality decreases.

Statistical analyses

To obtain an overall mean $T_{\rm e}$ for a given time period, we averaged $T_{\rm e}$ measured in each microhabitat. We then averaged all aquatic and all terrestrial microhabitats. Lastly, we took the mean of the aquatic and terrestrial habitats. We calculated a mean $T_{\rm b}$, $d_{\rm b}$, $d_{\rm e} - d_{\rm b}$, and $E_{\rm x}$ for each individual for each time period under consideration. Therefore, each individual is represented only once per time period (e.g., season) in all analyses. Data were not transformed because transformations did not improve normality. When the data were not normal, we used nonparametric tests. The assumption of homogeneity of variance was never violated. Significance of statistical tests was accepted at $\alpha = 0.05$. Values presented are means ± 1 SE. We ran all tests both with daily data and with daytime data only because painted turtles are diurnal. These tests gave quantitatively different, but qualitatively similar, results. We only report tests with the full data set.

Results

We implanted data loggers in 26 male turtles. We were unable to retrieve the implanted loggers from eight turtles. Predators killed three turtles and five turtles lost their transmitter. The remaining 18 turtles generated 31 297 measurements of $T_{\rm b}$ on 2 173 turtle-days.

Body temperature

We compared carapace temperature with internal $T_{\rm b}$ measured 5 cm in the cloaca on a fresh turtle carcass. The carapace and cloacal temperatures were only moderately correlated (r = 0.72). The residuals were from -12 to 17 °C. The carapace temperature (32.7 ± 0.81 °C) was significantly higher than the cloacal temperature (23.3 ± 0.83 °C) (paired *t* test: $t_{[106]} = 15.45$, p < 0.001).

For our 18 free-ranging turtles, distributions of $T_{\rm b}$ during daylight hours measured internally (with data loggers) and on the carapace (with temperature-sensitive radio transmitters) varied in their central 50%, mean, and range (Fig. 1). The 25th and 75th quartiles were 20.8 and 27.7 °C, respectively, for the transmitter-recorded temperatures, whereas they were 19.0 and 26.5 °C, respectively, for the internal $T_{\rm b}$. The transmitter-recorded temperature (24.3 \pm 0.26 °C) was significantly higher than the internal $T_{\rm b}$ (22.7 \pm 0.04 °C) (paired t test: $t_{[81629]} = 6.12$, p < 0.001). The maximum temperature recorded with the transmitter (41.6 $^{\circ}$ C) was above the critical thermal maximum for painted turtles (Hutchinson et al. 1966). Because of the disconnect between carapace temperature and internal $T_{\rm b}$ that we just demonstrated, we used $T_{\rm b}$ measured internally in all subsequent analyses.

Internal $T_{\rm b}$ recorded with data loggers throughout the day for the duration of the active season ranged from 7 to 39 °C (21.7 ± 0.14 °C). Measurements of $T_{\rm b}$ at 100 min intervals were not autocorrelated (ACF = 0.315, d = 1). Thus, we used the 100 min values as independent measurements.

Preferred body temperature

We took 420 $T_{\rm b}$ measurements from 15 male turtles in the thermal gradient. The $T_{\rm b}$ in the gradient ranged from 15.5 to 34.5 °C. The 25% and 75% quartiles averaged across individuals were 21.3 ± 0.53 °C and 25.0 ± 0.82 °C, and the mean $T_{\rm b}$ was 23.3 ± 0.59 °C. Thus, we considered $T_{\rm set}$ of male painted turtles to be 21.3–25.0 °C.

We wanted to compare T_{set} obtained in the laboratory with T_{b} selected in the field when T_{e} was permissive. We extracted measurements of T_{b} for time periods when the models indicated that T_{e} exceeded the lower bound of T_{set} (21.3 °C) in at least one microhabitat. Under these conditions, the field T_{b} was 23.3 ± 0.17 °C and the 25% and 75% quartiles were 20.5 ± 0.19 °C and 25.5 ± 0.22 °C, respectively. Temperatures selected in the thermal gradient and in the field when the environment was permissive were not significantly different (lower bound: $t_{[31]} = 1.03$, p =0.312; mean: $t_{[31]} = 0.05$, p = 0.958; upper bound: $t_{[31]} =$ 0.864, p = 0.394).

Operative environmental temperature

The copper models accurately estimated the T_b of a dead painted turtle. The temperatures of the model and of the car-

Fig. 1. Internal body temperatures and carapace temperatures measured during daylight hours throughout the active season of painted turtles (*Chrysemys picta*) in eastern Ontario. Carapace temperatures have a higher mean than internal body temperatures and some carapace temperatures were above the critical thermal maximum for the species.



cass were highly correlated (r = 0.979, m = 1.04, $F_{[1,2046]} = 47210$, p < 0.001), but the slope was significantly greater than one ($t_{[8188]} = 45.99$, p < 0.001). The mean difference in absolute value between model and turtle temperatures was significant (paired $t_{[4094]} = 5.819$, p < 0.001), but the difference ($0.98 \pm 0.02 \degree$ C) was less than the accuracy of the data loggers ($\pm 1 \degree$ C) and, thus, within measurement error. We assumed that the models accurately represented the operative environmental temperatures that were available to painted turtles.

We used air temperature, radiation, and wind speed to develop predictive equations of model temperatures in the four terrestrial microhabitats (Table 1). The correlation coefficient between the variables used in the regression equations was never more than 0.43. All equations had very good predictive power (all $R^2 > 0.73$; Table 1). We used these equations and climatic data to predict operative environmental temperatures for the duration of the active season.

Thermal quality of habitats

Throughout the active season, mean daily T_e ranged from 9 to 23.9 °C. Maximum T_e reached 67.5 °C and averaged 25.2 °C each day. Surface water temperature had the smallest mean d_e (1.4 °C), while rocks in the shade had the largest mean d_e (5.6 °C). Basking sites were as much as 44 °C above water temperatures. Mud temperatures never reached the lower bound of T_{set} and, thus, could be used as a refuge from high temperatures. For the entire active season, the mean thermal quality of the habitat (d_e) was 4.8 °C. Surface water temperatures were within T_{set} for 38.4% of the time. Basking site temperatures were within T_{set} for 16.7% ±

Microhabitat	Equation	R^2	р
Log			
Sun	1.45AIRT + 15.9RAD - 8.4649	0.83	< 0.0001
Shade	$0.99AIRT + 12.36RAD - 0.40WIND + 0.015AIRT^2 - 18.97RAD^2 + 0.05WIND^2 - 4.88$	0.73	< 0.0001
Rock			
Sun	0.93AIRT + 20.75RAD - 0.35WIND + 1.0998	0.73	< 0.0001
Shade	1.01AIRT + 13.16RAD - 0.28WIND - 3.5606	0.74	< 0.0001

Table 1. Predictive equations for painted turtle (Chrysemys picta) model temperatures in four locations.

Note: Equations were derived from air temperature (AIRT in °C), wind speed (WIND in m/s), and radiation (RAD in kW/m²) to predict terrestrial environmental operative temperatures (T_e).

Fig. 2. Mean hourly estimated operative environmental temperatures for water surface, pond bottom, basking site in the sun, and basking site in the shade during the active season of painted turtles in eastern Ontario. Horizontal solid lines represent the range in preferred body temperatures of painted turtles.



5.0% of the time and were above T_{set} for 21.1% ± 4.3% of the time (Fig. 2).

The mean monthly T_e increased from May to July, peaking at 21.4 °C, and then decreased from August until October. This indicated that in all months except July, mean T_e was not within T_{set} and, thus, the habitat available to painted turtles was thermally challenging.

General thermal ecology

The distribution of $T_{\rm b}$ and $T_{\rm e}$ for the active season indicated that painted turtles tended to select habitats that allowed them to be warmer, on average, than the environment and to avoid extreme temperatures. The mean $T_{\rm b}$ of painted turtles was higher than the mean $T_{\rm e}$ (21.6 vs. 18.9 °C) and the 25% and 75% quartiles were also higher (15.5 vs. 22.0 °C and 18.5 vs. 24.5 °C, respectively; Fig. 3).

We relocated the 18 turtles with radiotelemetry 548 times. We witnessed aerial basking on 15.7% of observations and aquatic basking (loafing partly submerged on aquatic vegetation) on 6.4% of observations. $T_{\rm b}$ was within $T_{\rm set}$ for 37.2% of observations, whereas mean available $T_{\rm e}$ was

Fig. 3. Frequency histograms of (A) the body temperature (T_b) of painted turtles and (B) the operative environmental temperatures (T_e) during the active season in a marsh in eastern Ontario. The shaded region represents the range in preferred body temperatures. The distribution of body temperatures compared with environmental temperatures indicates that turtles are avoiding the extreme temperatures.



within T_{set} for only 23.0% of observations (Fig. 3). We averaged distances moved weekly and regressed them against mean weekly d_{e} . There was no relationship between distance

moved and thermal quality of the environment ($R^2 = 0.009$, $F_{[1,262]} = 2.26$, p = 0.134).

Thermoregulation indices

The accuracy of body temperature (d_b) was 2.4 °C. Therefore, mean $d_e - d_b$ for the active season is 2.4 °C. We regressed d_b against mean d_e for each individual: the slopes ranged from 0.40 to 0.89 (0.58 ± 0.03) and R^2 was 0.25 ± 0.02 (Fig. 4).

We compared observations when T_{set} was available in at least one microhabitat and when it was unattainable. Turtles had higher $d_e - d_b$ when T_b within T_{set} could not be achieved (mean_{available} = 1.57 °C and mean_{unavailable} = 2.37 °C; Wilcoxon rank-sum test: Z = 9.52, p < 0.001).

 $T_{\rm set}$ was available in at least one of the microhabitats for 1415 observation periods, or 66.2% of the time during the active season. We calculated E_x for each individual and then averaged across individuals. $T_{\rm b}$ was within $T_{\rm set}$ for 40.7% \pm 1.3% of observations when $T_{\rm e}$ indicated that it was possible to maintain $T_{\rm b}$ within $T_{\rm set}$. $T_{\rm b}$ was below $T_{\rm set}$ for 31.6% \pm 1.8% of the time and above $T_{\rm set}$ for 27.7% \pm 1.2% of the time when $T_{\rm e}$ indicated that it was possible to maintain $T_{\rm b}$ within $T_{\rm set}$.

Season

We defined seasons by comparing mean daily air temperatures to the T_{set} bounds. The early season is from the beginning of the study until mean air temperature rose above the lower bound of T_{set} (16 May to 8 June). Mid-season is the period when mean air temperature is above the lower bound of T_{set} (9 June to 6 September). Late season is from when mean air temperature falls below the lower bound of T_{set} until the end of the study (7 September to 10 October).

A Kruskal–Wallis rank-sum test showed a significant difference in the effectiveness of thermoregulation $(d_e - d_b)$ among seasons $(\chi^2_{[2]} = 66.8, p < 0.001)$. Post hoc Wilcoxon rank-sum tests revealed that painted turtles invested more in thermoregulation in the early season (2.18 ± 0.05 °C) than in the mid-season (2.02 ± 0.02 °C) (Z = 6.97, p < 0.001), and less in late season (1.86 ± 0.03 °C) than in mid-season (Z = 3.30, p = 0.001). The magnitude of the seasonal differences, however, was very small.

For each season, we averaged hourly minimum and maximum $T_{\rm e}$, as well as hourly $T_{\rm b}$. We determined $E_{\rm x}$ for each individual for each season from hourly means. $E_{\rm x}$ across individuals was 41.1% ± 3.3% in early season, 40.4% ± 3.5% in mid-season, and 30.2% ± 5.3% in late season (Fig. 5). ANOVA showed no significant differences in $E_{\rm x}$ among seasons ($F_{12.491} = 2.06$, p = 0.14).

The frequency of basking behaviour differed by season (ANOVA: $F_{[2,500]} = 15.5$, p < 0.001). Tukey–Kramer HSD tests showed that there was significantly more basking in early season than in mid- or late season, but there was no difference in basking frequency between mid- and late season.

Discussion

Preferred body temperature

 T_{set} of painted turtles was previously estimated from distributions of T_{b} gathered from field-active individuals

Fig. 4. The absolute values of the difference between the body temperature and the range in preferred body temperatures (accuracy of body temperature: d_b) versus the absolute values of the difference between the environmental temperature and the range in preferred body temperatures (d_e) for painted turtles in eastern Ontario. Each turtle is represented by a solid gray line and the broken black line is the mean value. The solid black line represents the line of thermoconformity. Turtles are farther from thermoconformity when the thermal quality of the habitat is low.



captured opportunistically: 17-23 °C (Ernst 1972) and 20-25 °C (Brattstrom 1965). In a laboratory thermal gradient, we determined T_{set} to be 21.3–25 °C. The field values of $T_{\rm b}$ were not measured in an environment without costs. Thus, it is not surprising that the lower bound of field T_{set} is cooler than the lower bound of T_{set} determined in a laboratory thermal gradient because field $T_{\rm b}$ represents a compromise between the costs and the benefits of thermoregulation. The mean selected temperature of painted turtles (23.3 °C) in the thermal gradient was within the range of values reported for other species of turtles: 20.7 °C for Sternotherus odoratus (Latreille in Sonnini and Latreille, 1801) and 21.6 °C for Clemmys guttata (Schneider, 1792) (Graham and Hutchinson 1979); 28.1 °C for Chelydra serpentina (L., 1758) (Schuett and Gatten 1980); and 29.1 °C for *Pseudemys scripta* (= *Trachemys scripta* (Schoepff, 1792)) (Gatten 1974).

 T_{set} in painted turtles, and turtles in general, is low compared with that in other reptiles. Lizards have higher T_{set} . For example, T_{set} ranges 35.8–37.6 °C for Varanus panoptes Storr, 1980 and 34.0–36.3 °C for Varanus gouldii (Gray, 1838) (Christian and Weavers 1996); 33.9–37.2 °C for Sceloporus arenicolus Degenhardt and Jones, 1972; and 32.9– 38.3 °C for Uta stansburiana Baird and Girard, 1852 (Sartorius et al. 2002). Snakes also have a higher T_{set} than painted turtles. T_{set} in snakes usually ranges 28–34 °C (reviewed in Lillywhite 1987). Snakes in the same temperate

Fig. 5. Mean hourly body temperatures (solid black line) of painted turtles and maximum and minimum environmental temperatures (solid gray lines) in spring (A), summer (B), and fall (C). The range in preferred body temperatures of painted turtles is represented by the horizontal solid black lines.



study area have higher T_{set} : 24.7–29.8 °C for the northern water snake (*Nerodia sipedon* (L., 1758)) (Brown and Weatherhead 2000) and 26.5–29.8 °C for the black rat snake (*Elaphe obsoleta obsoleta* (Say in James, 1823)) (Blouin-Demers and Weatherhead 2001). Broad T_{set} values are typical for temperate species (5.1 °C for northern water snake, 3.3 °C for black rat snake, and 3.7 °C for painted turtle) because they experience large variations in T_{e} (Huey and Kingslover 1993).

Patterns in thermoregulatory effort

Standard indices of thermoregulation allow for comparisons between individuals within species and individuals among species. This is the first study to apply standard, quantitative indices of thermoregulation to turtles. Thus, no data are available on turtles for comparison. Previous methods of quantifying thermoregulation, such as length and frequency of basking bouts (Lefevre and Brooks 1995; Krawchuk and Brooks 1998), are not adequate because aerial basking is not the only means of thermoregulation. In this study, T_{set} was frequently available to turtles in aquatic microhabitats. Thus, aerial basking was not required to attain T_{set} in many instances. We showed that carapace temperature and $T_{\rm b}$ differ significantly in painted turtles. Because organismal performance depends on internal $T_{\rm b}$, not on carapace temperature, internal $T_{\rm b}$ is the metric under selection and should be used in studies of thermoregulation. Studies that measured carapace temperatures rather than internal $T_{\rm b}$ (e.g., Grayson and Dorcas 2004) overestimated basking temperatures and are, therefore, unreliable measures of thermoregulatory effort.

In painted turtles from Ontario, we found that the mean d_b (2.4 °C) was smaller than the mean d_e (4.8 °C) by 2.4 °C. The small d_e and d_b can be attributed partially to the wide T_{set} characteristic of the species, and not solely to small variations in T_b and T_e . Values of $d_e - d_b$ for lizards and snakes range from -0.33 to 8.4 °C (summarized in Blouin-Demers and Nadeau 2005). Compared with the documented range of $d_e - d_b$ for other reptiles, the painted turtles in this population can be classified as moderate thermoregulators. An assessment of how the effectiveness of thermoregulation in painted turtles compares with other turtles will have to await other quantitative estimates of thermoregulation in turtles.

 $E_{\rm x}$ also indicated that painted turtles are moderate thermoregulators. Painted turtles exploit the thermal environment and maintain $T_{\rm b}$ within $T_{\rm set}$ for 42% of the time when $T_{\rm e}$ is permissive. Northern water snakes in the same study area exploit the thermal environment and maintain $T_{\rm b}$ within $T_{\rm set}$ for 44% of the time when $T_{\rm e}$ is permissive (Brown and Weatherhead 2000). Black rat snakes in the same study area maintain $T_{\rm b}$ within $T_{\rm set}$ for 22.4% of the time when $T_{\rm e}$ is permissive (Blouin-Demers and Weatherhead 2001). Thus, other species in the same area have similar or lower $E_{\rm x}$ values.

Thermal quality of the habitat had no effect on movement patterns of painted turtles. Sexton (1959) suggested that the mechanism driving movement patterns in painted turtles is the cycle of macrophytes. The abundance of potential basking sites dispersed throughout the marsh indicates that movements were not constrained by the availability of thermoregulatory opportunities. Painted turtles in our study area appear to be able to follow the macrophyte cycle and maintain the opportunity to bask.

Season

The two indices of thermoregulation employed resulted in divergent conclusions about seasonal variation in thermoregulatory effort. The $d_e - d_b$ index detected significant albeit small seasonal differences, whereas none were detected with E_x . The former index established that thermoregulatory effort was highest in early season, lower in mid-season, and lowest in late season. Basking behaviour followed a similar pattern. Turtles basked more often earlier in the season than in mid- and late season. In early season, T_e was within T_{set} for less than half of the time than it was during mid-season. The early season was thus thermally challenging, and the turtles invested more in thermoregulation. Contrary to our predictions, the late season had the lowest thermal quality and the lowest investment in thermoregulation.

The late-season results may be a consequence of ecological constraints related to hibernation. Turtles cannot have food in their digestive system during hibernation because it could decompose owing to the cessation of digestion at low $T_{\rm b}$. To avoid this, painted turtles stop eating in late season (Sexton 1959). When there is no caloric intake, it is advantageous to maintain a lower metabolic rate to diminish energy expenditures. Thus, in late season, decreased thermoregulatory effort leading to lower $T_{\rm b}$ may be a consequence of the cessation of foraging in preparation for hibernation.

There was no difference in E_x across seasons, but in retrospect this finding is not surprising. E_x only examines thermoregulatory effort in a narrow range of temperatures: when T_{set} is available and the thermal quality of the habitat is, by definition, high. Constant E_x values across seasons demonstrate that turtles exploit the thermal environment to the same extent when exposed to the same thermal quality, independent of season.

The prediction of seasonal differences in thermoregulatory effort was based on the presumption that the mid-season would offer a more favourable thermal habitat than the early or late season. In fact, during all seasons high and low thermal quality habitats were available. The hourly mean variation in T_e was nearly as pronounced as the daily mean variation in T_e throughout the season. The average day had mean hourly T_e both inside and outside T_{set} . The mid-season did not always offer a better thermal environment than early or late season. Large variation in thermal quality within seasons renders seasonal comparisons less sensitive. Therefore, in this temperate habitat where variation in daily thermal quality is high, season may not be an appropriate proxy for thermal quality.

Thermoregulatory response to environmental temperatures

The thermal environment experienced by painted turtles in the study area was thermally challenging for most of the active season. Mean monthly T_e reached T_{set} in only 1 month. T_e was above the lower bound of T_{set} in basking sites only for 38% of the time. This is low compared with the common slider, a pond turtle found in South Carolina, where T_e in basking sites was above the lower bound of T_{set} for 61.5% of observations (Crawford et al. 1983).

We compared paired d_e and d_b values. When d_b was regressed on d_e , the slopes were between zero and one: the greater the value of d_e , (high d_e equals low thermal quality), the greater the difference between d_e and d_b (effectiveness of thermoregulation). Painted turtles raised T_b above T_e to a greater extent in low thermal quality habitats (high d_e). This is consistent with the pattern found by Blouin Demers and Nadeau (2005) among species of lizards and confirms the emerging pattern that reptiles invest more in thermoregulation when thermal quality is low.

Conclusion

This study confirmed that thermoregulatory effort within a population varies with thermal quality: painted turtles invest more in thermoregulation when thermal quality is poor. The next step is to investigate if populations living in habitats with different thermal quality invest differently in thermoregulation. Studies that have examined latitudinal variation in thermoregulatory effort without quantitative indices are equivocal. Ellner and Karasov (1993) found that a population of turtles at a northern location had lower T_b than a southern population. Andrews (1998) used a comparative approach among species and found that T_b was lower in the tropics than in northern temperate zones. Studies that quantify thermoregulation between populations remain scarce (for an exception see Diaz 1997).

Acknowledgements

We thank G. Bulté and S. Duscheneau for their able help with fieldwork. We are indebted to the staff at the Queen's University Biology Station for logistical support. A.L.E. is thankful to B. Young for his aid and support in the execution of this study. Funding for this research was provided by grants from the Natural Sciences and Engineering Research Council of Canada and the Canada Foundation for Innovation – Ontario Innovation Trust to G.B.-D.

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