Thermal environments of overwintering Eastern Box Turtles (*Terrapene carolina carolina*)

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Abstract: Using a mixed-effects model for 21 Eastern Box Turtles (*Terrapene carolina carolina* (L., 1758)) over three winters in Maryland, USA, we demonstrate that overwintering-site temperature was mainly related to air temperature. After controlling for air temperature, between-year variation accounted for 7%, between-turtle variation accounted for 3%, and variation owing to age class accounted for 1% of the total variation in overwintering-site temperature. Turtles showed overwintering-site fidelity and the location of overwintering sites did not depend on sex or age. According to the developed function, air temperature would have to increase by 3 °C over the overwintering period to raise the temperature of overwintering sites by about 1 °C, assuming no changes in other environmental factors; this level of warming is not expected until 2070–2090 according to general climate circulation models.

Résumé : L'utilisation d'un modèle à effets mixtes avec 21 tortues-boîtes de l'Est (*Terrapene carolina carolina* (L., 1758)) pendant trois hivers au Maryland, É.-U., nous a permis de démontrer que la température du site d'hivernage est surtout reliée à la température de l'air. Après avoir tenu compte de la température de l'air, la variation entre les années explique 7 % de la variation totale de la température dans le site d'hivernage; la variation entre les tortues explique 3 % et la classe d'âge 1 %. Les tortues montrent une fidélité au site d'hivernage et la position du site d'hivernage ne dépend ni du sexe, ni de l'âge. D'après la fonction obtenue, la température de l'air devrait augmenter de 3 °C durant la période d'hivernage pour accroître la température dans les sites d'hivernage d'environ 1 °C, en l'absence de changements dans les autres facteurs du milieu; un réchauffement de cette importance n'est pas prévu avant 2070–2090 d'après les modèles généraux de circulation du climat.

[Traduit par la Rédaction]

Introduction

Body temperature and environmental temperature are important factors that affect the growth, metabolism, and reproduction of turtles (Huey 1982; Congdon et al. 1989). As ectotherms, turtles produce insignificant internal heat and their body temperature is closely coupled to environmental temperature (e.g., Gates 1980). Body temperature is largely controlled through behavioral responses, which lead to the selection of favorable microclimates. Box turtles move through their home range during the active season, choosing favorable microsites (e.g., between sunny and shady locations, or between wet and dry sites).

Box turtles in temperate North America seek terrestrial overwintering sites to avoid the freezing and subfreezing air temperatures that are typical of winter in this area (Ultsch 2006). Subzero air temperature conditions are common across much of the range of the Eastern Box Turtle (*Terrapene carolina carolina* (L., 1758)) (Claussen et al. 1991). Thus, the turtles stay underground for 5–7 months a year, emerging in the spring when ambient environmental temperatures rise. Overwintering sites also provide protection from predators. Eastern Box Turtles typically overwinter in shallow burrows that they excavate in soft soil (Dodd 2001). They exhibit a high degree of site fidelity, often overwintering in the same area within their home range year after year (Dodd 2001). Forests with a thick layer of leaf litter are often selected for overwintering (Carpenter 1957).

A number of studies have documented the environmental conditions within overwintering sites of box turtles (Dolbeer 1971; Doroff and Keith 1990; Claussen et al. 1991; Storey et al. 1993). Claussen et al. (1991) used temperature-sensitive transmitters to monitor the microhabitat of overwintering Eastern Box Turtles in Ohio. They determined that it was not unusual for Eastern Box Turtles to experience body temperatures of –0.3 °C or below for several days each winter. Turtles were able to tolerate subfreezing temperatures and Claussen et al. (1991) concluded that winter survival is usually quite high. The Eastern Box Turtle is remarkable in its ability to tolerate body freezing and it is one of very few vertebrate species that can withstand freezing of a significant portion of its body (Costanzo and Claussen 1990; Claussen et al. 1991).

Whereas box turtles are well adapted to cold subsurface winter temperatures, warming air temperatures could cause box turtles to enter overwintering sites later in the fall and to emerge earlier in the spring. However, a reduction in the number of days spent underground in winter, safe from most mortality factors, could be stressful to long-lived animals

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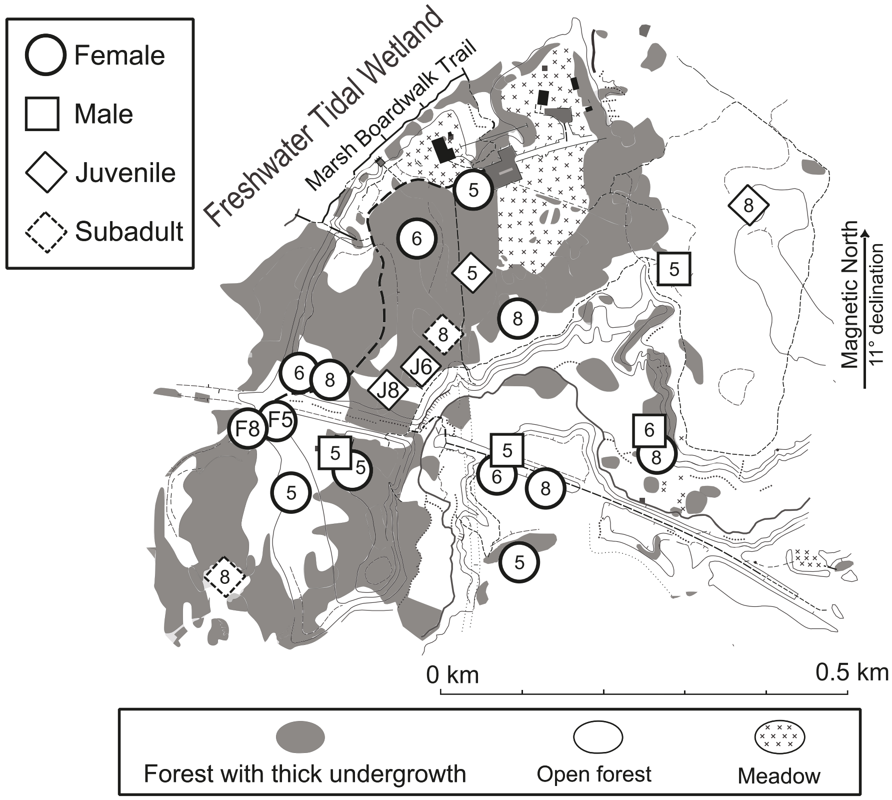
Fig. 1. Locations of overwintering Eastern Box Turtles (*Terrapene carolina carolina*) studied over three winters at Jug Bay Wetlands Sanctuary, Maryland, USA. F5 and F8 were the same female; J6 and J8 were the same juvenile. The numbers 5, 6, and 8 inside the symbols indicate winters of 2004–2005, 2005–2006, and 2007–2008, respectively.



that remain inactive underground for a large proportion of their life. Converse et al. (2005) demonstrated that the highest survival rates for Ornate Box Turtles (*Terrapene ornata* (Agassiz, 1857)) would be expected in years with low minimum winter temperatures, and they speculated that if box turtles experience higher overwintering temperatures owing to global warming, their metabolism may increase enough to cause lipid stores to be used up prior to spring emergence. Another study suggested that the degree of climate warming that is predicted by 2100 might decrease the probability of hatchling Eastern Box Turtles growing during their first year to <20%, and would result in earlier termination of adult growth, smaller carapace lengths, and reduced fecundity (McCallum et al. 2009).

Our study was initiated because of the dirth of studies on the thermal environments of overwintering box turtles. The main objective of the study was to analyze the variability in daily temperature of overwintering sites in relationship to air temperatures, age classes, and individual turtles. More specifically, we wanted to (*i*) test if the location of overwintering sites varied by age or sex classes; (*ii*) estimate the effect of the mean daily air temperature on the mean daily temperature of overwintering sites of turtles; and (*iii*) estimate variability in overwintering-site temperature related to differences in age classes and individual turtles.

We hypothesized that daily variation in air temperature is the main factor affecting daily variations in temperature of the overwintering sites. This is because soil temperature is strongly influenced by air temperature (e.g., Savva et al. 2010), and in the winter turtles have no internal heat production, have very low metabolism, and are inactive (Dodd 2001). We also predicted that juveniles and adults might select overwintering microsites with differing thermal properties owing to differences in their body mass and possible physiological needs.

Materials and methods

Study site

The study site was located in southern Anne Arundel County, Maryland, USA (38.8°N, 76.7°W; Fig. 1), within the Jug Bay Wetlands Sanctuary. The 53 ha study site included forests, managed meadows, tidal and nontidal wetlands, and permanent streams along the tidal Patuxent River estuary. All turtles overwintered in dry, well-drained, sandy soils within an upland forest that was dominated by deciduous hardwood trees including southern red oak (*Quercus falcata* Michx.), American beech (*Fagus grandifolia* Ehrh.), yellow poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), and by the conifers Virginia pine (*Pinus virginiana* P. Mill.) and eastern redcedar (*Juniperus virginiana* L.). To track turtles to overwintering sites, in summer we attached 5 g Holohil Ltd. brand transmitters to the rear carapace using PC7 epoxy glue. Turtles were tracked with an Advanced Telemetry Systems R1000 receiver to determine the dates when they entered their overwintering sites. Tur-

Table 1. Characteristics of the Eastern Box Turtles (*Terrapene carolina carolina*) overwintering during the three studied winters in the Jug Bay area, Maryland, USA.

					Hourly and two-hourly overwintering-site temperature (°C) from 18 Dec. to 6 Apr.			
Turtle ID No.	Sex	Body mass (g)	Carapace length (mm)	First day of overwintering	Mean	SD	Minimum	Maximum
2005								
53	Female	285	119	25 Oct.	4.4	2.6	0.5	13.5
64	Male	415	133	20 Oct.	4.9	2.6	1	14
100	Female	372	117	8 Nov.	4.7	3	0.5	17.5
235	Male	391	136	8 Nov.	4.1	3	0	15
266	Male	365	128	22 Nov.	4.6*	2.3*	1.5	13.5*
319	Female	382	132	1 Nov.	4.4	2.41	–0.5	12.5
370	Female	440	126	16 Nov.	4.3	3.2	–1	16.5
383	Female	334	114	16 Nov.	5.1	2.4	1	16.5
437	Juvenile	120	87	15 Nov.	4	3.2	–0.5	14.5
Mean		345	121	5 Nov.	4.5	2.8	0.3	15
Air temperature					2.9	6.5	–12.8	28.9
2006								
5	Female	448	137	23 Nov.	5.4	3	0.5	15.5
45	Female	370	123	23 Nov.	5.9	2.2	2.5	12.5
112	Male	280	118	10 Nov.	6.3	2.6	2	16
336	Female	453	131	23 Nov.	5*	2*	0.5	11.5*
458	Juvenile	62	72	27 Oct.	5.5	2.7	1	16.5
Mean		323	116	15 Nov.	5.8	2.6	1.3	15.1
Air temperature					5.4	6	–10.6	28.3
2008								
151	Female	430	130	14 Nov.	6.8	2.3	2.2	12.9
226	Female	400	127	28 Oct.	7.5	1.4	4.6	10.7
248	Female	410	127	4 Nov.	6.2	1.9	2.4	11.3
319	Female	450	137	21 Nov.	7.5	1.8	4.1	11.9
322	Female	492	132	29 Nov.	6.6	2.1	2.7	12.2
350	Subadult	300	122	16 Nov.	7.8	2	3.8	12.9
457	Subadult	334	119	2 Nov.	7	1.8	3.6	11.7
458	Juvenile	77	77	2 Nov.	5.3*	1.9*	2.2*	11*
498	Juvenile	111	83	2 Nov.	7	2.2	2.8	13
Mean		334	117	9 Nov.	7.1	1.9	3.3	12.1
Air temperature					5.3	6.3	–10.7	23.7

Note: The period from 18 December to 6 April was chosen to ensure full data coverage for most turtles (i.e., absence of missing values) to provide unbiased comparisons across the three winters. According to our measurements, hourly observations are shown for 2008 and two-hourly observations for 2005 and 2006. Body mass and carapace length were measured before entering the overwintering sites.

*Value measured for a shorter period of time than the other temperature measurements.

tles exited the overwintering sites in April. Our observations of exiting dates were approximate with an error of ±10 days because we were not able to visit every turtle every day. Thus, these data were not used in the analyses. The depth of turtles was checked by running a metal ruler through the soft soil to the top of the carapace. The overwintering sites were visited monthly over the winter to make certain that the turtles did not move to a different overwintering site.

Turtle age and sex classes

For this study, turtles were grouped into three age classes based on carapace length: juveniles, subadults, and adults. Turtles with a straight-line carapace length less than 107 mm were considered juveniles, those 107–120 mm were considered subadults, and those over 120 mm were considered adults (see Hall et al. 1999). Adults were classified into two sex classes (females and males) by examining external morphological characteristics: presence of a plastron depression; tail length; cloaca position in relation to posterior margin of the carapace; and hind-claw shape (Dodd 2001).

Overwintering-site and air temperatures

Temperatures of the overwintering sites were collected from November to April. The data were combined from the three independent experiments to ensure a high level of replication. Overwintering-site temperatures were measured with iButtons (Maxim Integrated Products, Inc.) in 2005–2006 (nine turtles) and 2006–2007 (five turtles), and with the wireless sensor networks (WSN) in 2008–2009 (nine turtles) (Table 1). Two of the same turtles from this data set were studied over two winters.

The iButtons were glued to the posterior of the turtle's carapace and recorded temperature to the nearest 0.5 °C every 2 h. Considering that turtles can move downward in the soil when temperature drops and upward when temperature rises, iButtons can measure temperature at the actual overwintering depths. In 2008–2009, the temperature of overwintering sites was measured every 30 min with 0.02 °C precision using temperature sensors from WSN developed in our laboratory at Johns Hopkins University (http://www.lifeunderyourfeet.org). The sensors were placed at 10 cm soil depth, which we considered a reasonable estimate for depth of overwintering Eastern Box Turtles based on personal experience and prior studies (e.g., Claussen et al. 1991; Ultsch 2006). About 5% of the measurements for some turtles were missing owing to occasional sensor failures.

Mean daily air temperatures for Baltimore–Washington International (BWI) Airport (39°10′N, 76°41′W; 47.5 m above sea level) located about 55 km from the study site were obtained for the period 2004–2009 (National Climatic Data Center 2008). January is the coldest month in the area with the mean daily air temperature of –0.1 °C. Mean November air temperature at the onset of the overwintering period was 8.2 °C. Mean April air temperature when turtles typically exit the overwintering site was 11.8 °C. Mean total precipitation from November to April was 496 mm. Considering that the overwintering sites were located in a forested area and were about 55 km away from the weather station, the air temperature at the overwintering sites might differ slightly from the air temperature recorded at the BWI weather station. Also this weather station had the minimal number of missing values over the studied period compared with other weather stations in the area.

Soil temperature model development

For this analysis, we used the mean daily measurements of overwintering-site temperature. Only 1 year of data was used for each turtle, i.e., for turtle No. 319 only in 2005 and for turtle No. 458 only in 2008. The structure of the data allowed using a mixed-effect model to test for the effect of the mean daily air temperature on the mean daily temperature of the overwintering sites, from November to April, over the 3 years. A mixed-effects model was used rather than a fixed-effects model because it could separate the random variation (owing to differences in measurement techniques, age classes, individual turtles, and other unaccounted between-year factors) from the variation owing to air temperature. Overwintering-site temperature exhibited a cyclic pattern that followed the pattern of air temperature (Fig. 2). Therefore, a trigonometric function was used to account for cyclic variations in the overwintering data. A few functions were tested such as the sine and cosine waves alone, but the model containing both Fourier terms (sine and cosine) was a better fit. The variance of residuals, Akaike's information criterion (AIC) (Sakamoto et al. 1986), Bayesian information criterion (BIC) (Schwarz 1978), and log-likelihood (Searle et al. 1992) were used as criteria for selecting the best model.

The intercept, amplitude, and frequency of the wave were used as fixed effects in the model. The mean daily air temperature was tested as a covariate to them. The years, age classes (adults, subadults, and juveniles) within years, and the overwintering turtles within age classes within years were tested as random effects to the intercept, slope, and wave amplitude. Thus, the random effects estimated the variation in overwintering-site temperature owing to differences between environmental and microenvironmental conditions and the temperature measurement techniques between the years (i.e., year effect), age classes (i.e., age class nested within years effect), and individual turtles (i.e., turtles nested in age class in years effect).

The covariate was tested for exclusion by using AIC, BIC, and likelihood ratio tests. The final mixed-effects model included only significant terms, the significances of which were tested using the Wald tests (Agresti 1996). The relative variability of the random effects was calculated as ratios of standard deviations (SD) of the random effects to the absolute values of the corresponding fixed effects. The random effects with relative variabilities above zero were only kept in the final model. The final mixed-effects model of the mean daily temperature of the overwintering site $T\text{site}_{iksj}$ (°C) for the kth year of the sth age class (adults, subadults, and juveniles), jth turtle for the ith day at time t_{iksj} (ranging from 1 to 366 days) at the mean air daily temperature $T\text{air}_i$ (°C) is

$$[1]\quad T\text{site}_{iksj} = (\phi_0 + B_0 T\text{air}_i + c_{0k} + c_{0k,s} + c_{0k,s,j}) + (\phi_1 + B_1 T\text{air}_i)\sin\frac{2\pi}{365}\phi_3 t_{iksj} + \phi_2\cos\frac{2\pi}{365}\phi_3 t_{iksj} + e_{iksj}$$
$$c_{0k} \sim N(0,\sigma_1^2),\ c_{0k,s} \sim N(0,\sigma_2^2),\ c_{0k,s,j} \sim N(0,\sigma_3^2),\ e_{\text{iksj}} \sim N(0,\sigma^2)$$

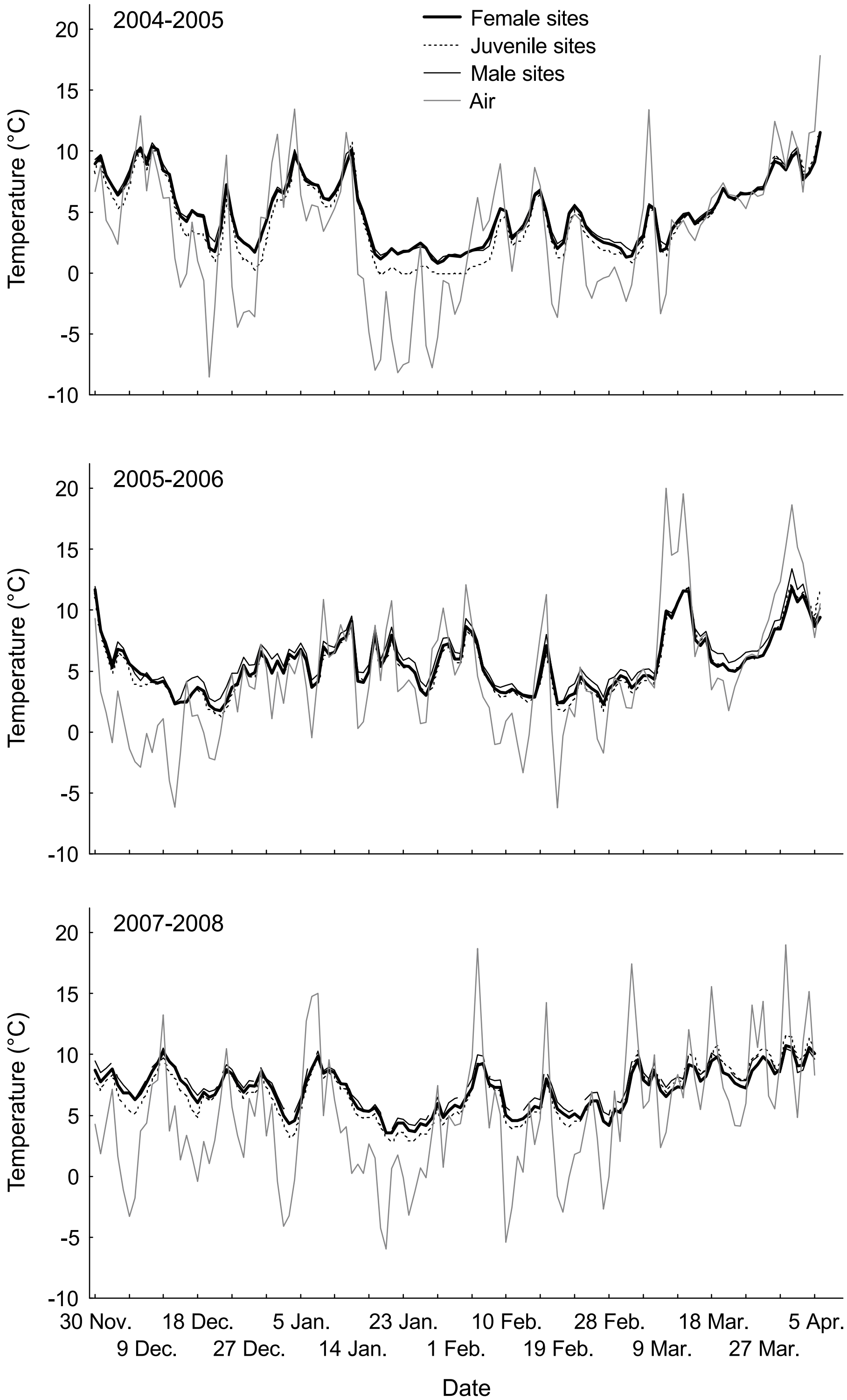
where ϕ_0 (intercept), ϕ_1 and ϕ_2 (amplitude components), and ϕ_3 (frequency) are the fixed effects, and B_0 and B_1 are main effects of the mean daily air temperature. The random effects c_{0k}, $c_{0k,s}$, and $c_{0k,s,j}$ are assumed to be independent for different k, s, and j. The within-group error, e_{iksj} is assumed to be independent for different i, k, s, and j and independent of the random effects.

Considering that overwintering site temperature on one day was dependent on the previous day's overwintering-site temperature, we used the mixed autoregressive–moving average model (ARMA) (1,1) to model dependence among the within-group errors (Box et al. 1994):

$$[2]\quad e_{ikjs} = \rho e_{(i-1)ksj} + \theta\alpha_{(i-1)ksj} + \alpha_{iksj}$$
$$a_{iksj} \sim N(0,\sigma_a^2 I)$$

where there are two correlation parameters ρ and θ. This correlation function for ARMA(1,1) behaves like the correlation function of an autoregressive model (AR) (1) for lags greater than one and like an AR(1) correlation function plus

Fig. 2. Variation in mean daily air and overwintering-site temperatures during the three study winters for 21 Eastern Box Turtles (*Terrapene carolina carolina*).



an additional term related to the moving average part of the model for lag 1. The α_{iksj} values are independent and the error term $E[\alpha_{iksj}] = 0$. This model described the dependence of the residuals better than AR(1) and MA(2) models. The two models were compared using the likelihood ratio test, AIC, and BIC.

Differences in geographical locations of overwintering sites between age and sex classes

Differences and similarities in the geographical coordinates (latitude and longitude) of overwintering sites between the three age classes (adults, subadults, and juveniles) and the two sexes were analyzed using Ward's multivariate tech-

Fig. 3. Cluster diagram of the geographical coordinates of overwintering Eastern Box Turtles (*Terrapene carolina carolina*). Turtle number incorporates the ID number from Table 1, age or sex class, and year. F, M, S, and J denote female, male, subadult, and juvenile, respectively. The numbers 5, 6, and 8 indicate the winters of 2004–2005, 2005–2006, and 2007–2008, respectively.

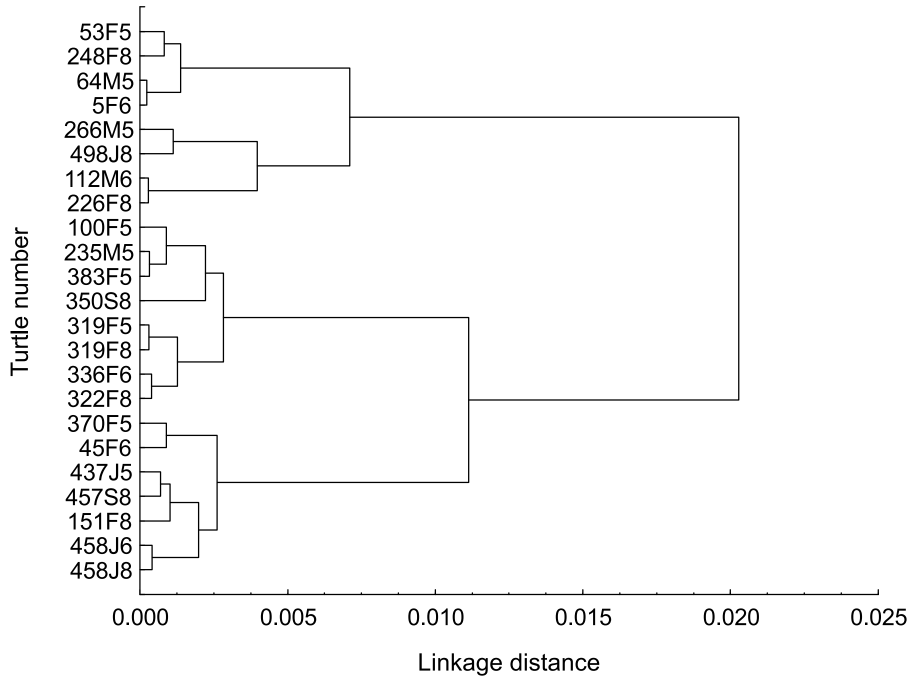


Table 2. Parameters of the model for overwintering-site temperature of Eastern Box Turtles (*Terrapene carolina carolina*) in Jug Bay, Maryland, USA.

Term	Value	SE	95% confidence interval	Parameter description
Fixed effects				
ϕ_0	10.0***	0.48		Intercept
B_0	0.3***	0.01		
ϕ_1	–3.1***	0.25		Sine amplitude
B_1	–0.1***	0.01		
ϕ_2	–5.2***	0.26		Cosine amplitude
ϕ_3	1.0***	0.01		Frequency
Estimates of autocorrelation model				
ρ	0.74		0.70–0.77	Autoregressive parameter
θ	0.27		0.23–0.32	Moving average parameter
Standard deviations of random effects				
c_{0k}	0.70			Random effect estimates for intercept
$c_{0k,s}$	0.13			
$c_{0k,s,j}$	0.34			
Residuals	1.39			

Note: See the Materials and methods for explanations of abbreviations of terms. ***, $p < 0.0001$.

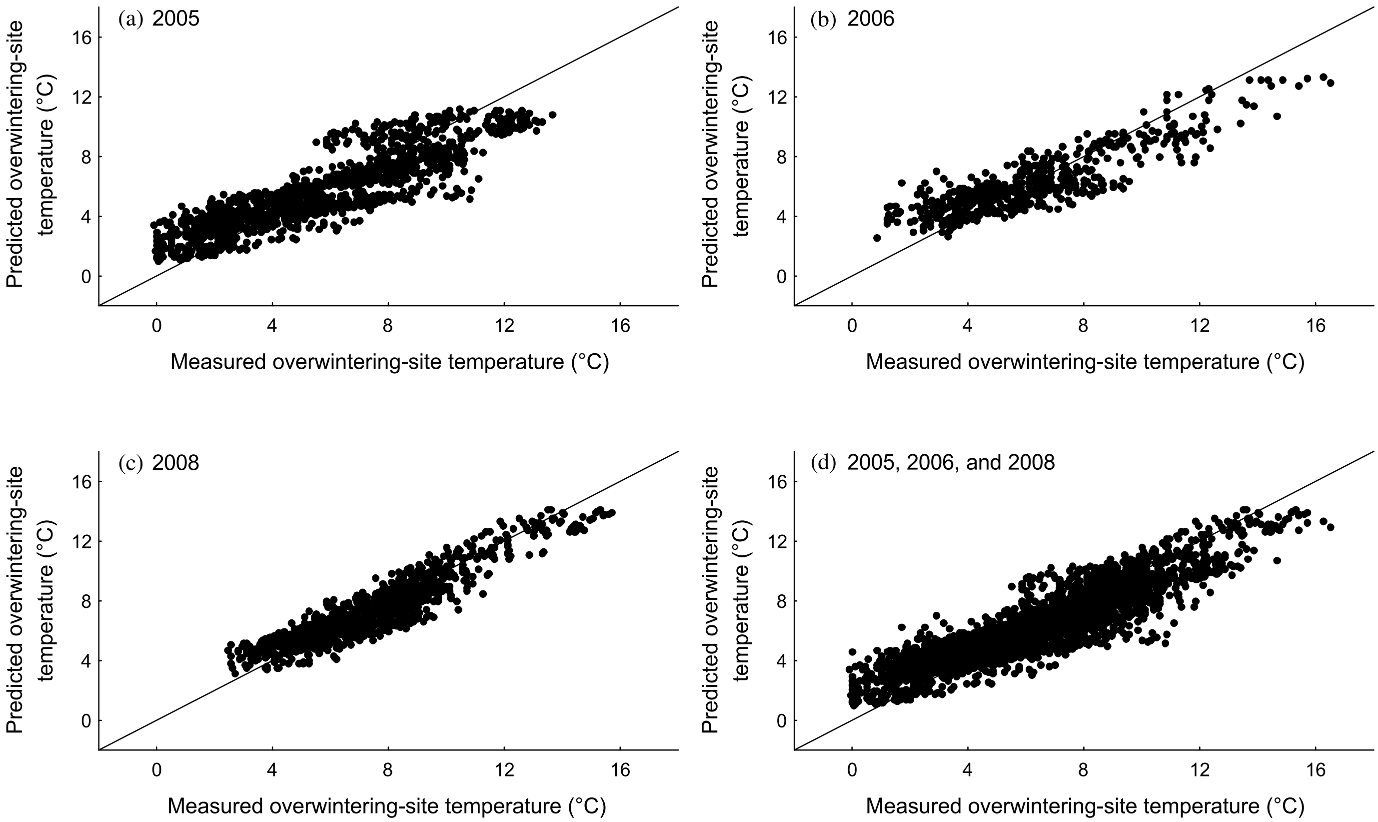
nique to identify those with maximum similarities within each cluster and the distance between the obtained clusters (Anderberg 1973). The analyses were performed using the R and nlme software in R version 2.8.1 (Pinheiro and Bates 2000; R Development Core Team 2007).

Results

Sex- and age-related differences in selection of overwintering sites

The overwintering sites were separated into three geographic clusters, but there was no pattern in their clustering related to age, sex, or overwintering year (Fig. 3). Each of the three clusters included adults and a juvenile or a subadult. Therefore, the juveniles, subadults, males, and females did not stand as separate classes, suggesting that the location of the overwintering sites did not depend upon the sex or age class. Also, a juvenile (No. 437J) was located more closely to a subadult and a female than to another juvenile. Similarly, juvenile No. 498 was found closer to adults than to other juveniles. Our observations and the cluster analysis suggested that Easter Box Turtles tended to return to almost

Fig. 4. Predicted versus observed values of the daily overwintering-site temperatures of Eastern Box Turtles (*Terrapene carolina carolina*) plotted separately (*a*, *b*, *c*) and combined for the three study winters (*d*). The solid line indicates a 1:1 ratio.



the same site to overwinter each year (e.g., No. 458J and No. 319F in Fig. 3).

Effect of air temperature on overwintering-site temperature

The model fitted the observed values of soil temperature with a high degree of accuracy (Table 2, Fig. 4). The mixed-effects model demonstrated significant effects of air temperature on overwintering-site temperature over the study period from November to April (Table 2). The effect was negative to the amplitude of the wave (–3.1), demonstrating that the wave of overwintering-site temperature is reduced over the *y* axis compared with the wave of air temperature; i.e., the overwintering-site temperature was lower at high values of air temperature and it was higher at low values of air temperature (Fig. 2). This is because of the presence of a conducting layer with relatively high heat capacity (i.e., soil and leaf litter) between the air and the turtles. Additionally, there was a positive effect of air temperature as an intercept to the wave, demonstrating a positive effect on the mean temperature of the sites (Table 2).

According to our developed function (Table 2; eqs. 1 and 2), if the mean air temperature over the studied period increased by 1 °C, holding other variables constant, the overwintering-site temperature would increase by 0.3 °C. Our model predicts that to increase the overwintering-site temperature by about 1 °C over the period from November to April, the mean air temperature would have to increase by about 3 °C over the same period.

At below-freezing air temperatures when the daily air temperature dropped, on average, to –2.4 °C, the daily overwintering-site temperatures were always above freezing (3.4 °C, on average, for adults and 3 °C for juveniles) except for two turtles for which the minimum temperature decreased to –0.5 °C one day over the studied period.

Between-year, age class, and individual turtle differences in overwintering-site temperature

Variance components related to random effects were above zero only for the intercept and were close to zero for the amplitude and the frequency of the wave (Table 2). Random variation among the years explained 7% of the total variation, random variation among the age classes within the years explained 1%, and random variation between individual turtles within the age classes within the years explained 3% of the total variation relative to the fixed effect.

Discussion

Our study demonstrated a significant effect of the daily air temperature on daily temperature of overwintering sites of Eastern Box Turtles. The importance of an insulating layer of leaf litter, forest understory, and soil between the cold air and the turtle is demonstrated by the reduction of the wave of overwintering-site temperatures over the *y* axis relative to the wave of air temperatures. Sandy soils are well drained, have a low surface area per gram of soil, and high porosity. Thus, their water-holding capacity is lower compared with other soil types (e.g., Roberts et al. 2006). In spite of the high thermal conductivity of water, the low water-holding capacity of sand does not allow for rapid heat flow through the sandy soil layers. We suggest that this is why, despite freezing air temperatures, the overwintering-site temperatures were always above freezing, except for two turtles, for which the minimum temperature decreased to –0.5 °C one day over the studied period. This demonstrates that the overwintering-site temperature in the Jug Bay area is not near the Eastern Box Turtle's minimal thermal limits. The Eastern Box Turtle has a remarkable ability to tolerate freezing, with ice penetration throughout the body cavity and ice contents that can reach equilibrium values of over 50% of total body water (Storey and Storey 2004). Adult box turtles, at about 0.5 kg body mass, are the largest freeze-tolerant animal (Storey 2006). Laboratory experiments demonstrated the ability of turtles to recover from body temperatures as low as –3.6 °C for up to 3–4 days, with 44%–58% of their body water frozen (Costanzo and Claussen 1990; Storey et al. 1993).

Our study demonstrated that 7% of the variation in overwintering-site temperature was related to differences between study years. This variation included differences in other environmental factors not accounted by the model, which varied between the years. These factors may have included differences in the mass and quality of leaf litter, precipitation, and the measurement techniques between the studied years. For example, intercept coefficients for these random effects were –0.71, –0.19, and 0.90 for winters of 2005, 2006, and 2008, respectively. The coefficients did not differ much between the years 2005 and 2006; differences were higher between these years and 2008. It might be because in 2005 and 2006 the iButton sensors were fixed to the turtles' shells, and thus measured the overwintering-site temperature more accurately (i.e., at actual overwintering depths), but the measured fixed depth in 2008 might have been slightly deeper than the real depth of the overwintering sites. Yet, the differences in these techniques did not bias our results, because the mixed-effects model separated this random variation owing to the technique differences from the fixed effect of our interest (i.e., air temperature) and the majority of our turtles were measured at real overwintering depths (i.e., using the sensors fixed to the shells).

Variability in overwintering-site temperature between the age classes (adults, juveniles, and subadults) was only 1%. This demonstrated that there were almost no age-related differences in the overwintering-site temperature.

Individual turtle variability in overwintering-site temperature was also low (3%). We attribute this low variation to microenvironmental differences between the overwintering sites selected by the turtles; i.e., differences in topography, water flow into the soil, quality and mass of leaf litter between the overwintering sites, and overwintering depth within age classes within years.

Results of the model were not biased by several days of snow cover because there were only 18 days with precipitation above zero during the days when the air temperature decreased below freezing over the three studied winters. The median amount of precipitation was only 0.5 cm during the 18 days and snow cover did not persist for long. The 0.25 quintile of the mean daily air temperature was 1.09 °C and the zero quintile was –8.5 °C over the studied period. Furthermore, for these days with negative daily air temperature and precipitation above zero, the SD of the residuals was not higher (1.02 °C) than that of all residuals over the studied winters (1.39 °C; see Table 2).

Although there were no differences between age or sex classes in locations of overwintering sites, the two turtles that we observed over 2 years returned to the same overwintering sites in the following year. This finding is consistent with those for other Eastern Box Turtles in Maryland (Hall et al. 1999) and for Ornate Box Turtles in Nebraska, USA (Converse et al. 2002), confirming that box turtles often return to the same area to overwinter (Dodd 2001). Considering that turtles exhibit strong site-fidelity behavior in spite of large differences in air and overwintering-site temperatures between the years, we suggest that turtles might be limited in their ability to move to new overwintering sites in response to increases in air temperature.

According to our derived function (Table 2; eqs. 1 and 2), an increase in the mean air temperature over the studied period by 1 °C should increase the mean overwintering-site temperature over the study period by 0.3 °C and an increase in mean air temperature by about 3 °C should increase the mean overwintering-site temperature by about 1 °C (holding other factors constant). The latter scenario is not projected to occur until 2070–2090, according to current general circulation climate models such as integration HadCM2GGA1 of the HadCM2 model (Cullen 1993) and scenario CCGSA3 of the Canadian Global Coupled Climate Model (CGCM1) (McFarlane et al. 1992; Flato et al. 2000). Higher overwintering temperatures may cause physiological stress by increasing metabolism, thus causing stored lipids to be used up prior to spring emergence as suggested by Converse et al. (2005). Also, it has been shown recently for Three-toed Box Turtles (*Terrapene carolina triunguis* (Agassiz, 1857)) in Arkansas, USA, that the temperature increase in winter (and decrease in spring) can significantly decrease turtle growth (McCallum et al. 2009). As the climate warms, turtles might also enter overwintering sites later in the season and emerge earlier in the spring, and thus be exposed longer to food scarcity, predation, natural disasters such as floods or fire, or to anthropogenic impacts such as vehicle crushing on roads. Populations of box turtles are declining throughout much of their range in North America (Dodd 2001). Climate warming therefore may become an additional factor that exacerbates these declines.

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