

Thermal environment and microhabitat of ornate box turtle hibernacula

Joseph R. Milanovich, Brock P. Struecker, Stanislaw A. Warcholek and Leigh Anne Harden

J. R. Milanovich (jmilanovich@luc.edu), B. P. Struecker, S. A. Warcholek and L. A. Harden, Dept of Biology, Loyola Univ. Chicago, 1050 W. Sheridan, Chicago, IL, USA. Present address for SAW: College of Veterinary Medicine, Kansas State Univ., Manhattan, KS, USA. Present address for LAH: Dept of Biological Sciences, Benedictine Univ., Lisle, IL, USA

Box turtle populations are under significant threat from historic and current alterations of land-use where remaining populations often occur in remnant patches of suitable habitat surrounded by a heterogeneous mixture of anthropogenically altered, unsuitable habitat. Ornate box turtles *Terrapene ornata* are a prairie-dependent species, are considered Near Threatened by the International Union for Conservation of Nature (IUCN), and are Endangered or Threatened within many US states. Since their range exists largely in northern latitudes, aspects of hibernacula are particularly important for winter survival. How box turtles select hibernacula within prairies is likely of great importance to better understand which microhabitats are selected for and to identify areas of suitable habitat for management purposes. Using radio telemetry in conjunction with temperature dataloggers (iButtons), we examined the phenology and thermal characteristics of box turtle hibernation, and quantified microhabitat selection of their hibernacula in remnant prairie patches. We monitored hibernation initiation/termination phenology and turtle carapace temperatures, and quantified vegetative and soil microhabitat variables of hibernacula for seven turtles in 2014/2015 and 18 turtles in 2015/2016. Box turtles initiated (descended into hibernacula) and terminated (ascended out of hibernacula) at similar time periods across two years. Although the ambient thermal environment consistently experienced temperatures below freezing, turtle hibernacula offered a buffer against those temperatures and thus the temperature of turtle carapaces never fell below freezing. Turtles selected microhabitat hibernacula with higher percentages of sand, leaf litter and bare ground, and lower percentages of clay, silt, shrubs and herbaceous ground cover. Our study suggests the phenology of box turtle hibernation may be similar across years and that hibernacula selection is driven by above- and belowground characteristics that ultimately lead to a more stable and warmer thermal environment.

Native prairie habitats in the Midwestern United States (US) have declined in area up to 99.9% since European settlement. Prairies once covered over 65 million hectares in the US, whereas they now occur in only remnant patches across the Midwest (Samson and Knopf 1994). The decline has been attributed to fire suppression, conversion to agriculture and removal of keystone species (e.g. American bison *Bison bison*; reviewed by Samson and Knopf 1994). Many populations of prairie-dependent species have been decimated to the point of threatened or endangered status as a consequence of the decline in native prairies (Pergams and Nyberg 2001, Brennan and Kuvlesky 2005, Ceballos et al. 2010). These species have specific life history requirements centered around using specific habitats across their yearly phenology (e.g. hibernacula) that, if identified, could be used to most

effectively develop management plans for native or restored prairie ecosystems.

Winter, in particular, is a critical period to the life history and survival of northern species and often species require specific habitats to survive long periods of unsuitable temperatures via hibernation. Poikilotherms (e.g. herpetofauna) in northern latitude prairie ecosystems, for example, may withdraw into hibernacula for eight to eleven months (Breckenridge and Tester 1961, Zug et al. 2001, Converse et al. 2002, Mathew et al. 2006). Thus, timing of initiation and termination of hibernation is imperative to offer proper protection for winter survival. In northern latitudes, the phenology of hibernacula initiation and spring termination are therefore similar for many prairie-dependent herpetofauna including snakes (Graves and Duvall 1990, Mathew et al. 2006), frogs (Willis et al. 1956, Cunjak 1986) and aquatic turtles (Edge et al. 2009). Hibernation is particularly important for poikilotherms whose body temperatures are regulated by the physical environment. Herpetofauna utilize several mechanisms to help avoid mortality during cold winter conditions, including thermogenesis, sheltering,

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hibernation, freeze tolerance and freeze avoidance (reviewed by Storey and Storey 2001).

Selection of herpetofauna hibernacula can be related to specific microhabitat variables (Ultsch 1989, Claussen et al. 1991, Thienpont et al. 2004, Steen et al. 2007, Harden et al. 2009) and may be important to facilitate ease of burrowing and to provide thermal protection for turtles. Sandy soils facilitate burrowing in a number of vertebrate taxa such as pocket gophers *Thomomys botae* where energetic cost of burrowing in sandy soils is far less than clay (Vleck 1981). Additionally, gopher tortoise *Gopherus polyphemus* burrows are positively associated with sandy soils (Jones and Dorr 2004). In northern latitudes, poikilotherms must remain below frost line in addition to having cold-adaptive strategies during hibernation. Several studies have found box turtles can regulate body temperature in hibernacula by actively adjusting their depth to maximize heat exposure and to minimize freezing during hibernation (Carpenter 1957, do Amaral et al. 2002, Currylow et al. 2013), which supports the potential selection of sandy soils that are easier to traverse compared to more compacted soils. Examining the extent to which these habitats offer thermal protection will provide valuable information on the life history of northern latitude prairie-dependent wildlife species. In turn, these data can be used to prioritize habitats to include in restored prairie ecosystems as well as to manage restoration practices (e.g. revegetation, prescribed fires).

This study provides data on thermal environment and habitat selection for hibernacula of the ornate box turtle *Terrapene ornata*, a prairie-dependent species threatened across much of its current range (Doroff and Keith 1990, Dodd 2002, Ernst and Lovich 2009). Across Midwestern prairie ecosystems of the US, ornate box turtles were historically common, but now occur in isolated remnant patches due to severe habitat loss and alteration (Smith 1961, Ernst and Lovich 2009). Box turtles use a variety of habitats for feeding, reproduction, thermoregulation and hibernation (Ernst and Lovich 2009). However, we know relatively little regarding how ornate box turtles select hibernacula. These hibernacula are likely of considerable importance, as tortoises can hibernate for up to seven months (Doroff and Keith 1990, Díaz-Paniagua et al. 1995, Converse et al. 2002, Bernstein and Black 2005) in northern latitudes and these habitats must provide protection from the external environment.

Box turtle species have freeze tolerance capabilities (Costanzo and Claussen 1990, Costanzo et al. 1995). However, mortality during winter months, likely due to freezing, is not uncommon (Metcalf and Metcalf 1979). Turtles require hibernacula locations that are suitable to burrow and that will provide a thermal environment necessary for survival. Minimal data exists regarding the specifics of hibernacula microhabitat selection of ornate box turtles, but studies suggest hibernacula occur primarily in loose sandy soil and in common blackberry *Rubus allegheniensis* thickets (Metcalf and Metcalf 1970, 1979, Doroff and Keith 1990, Converse et al. 2002, Bernstein and Black 2005). However, there has not been a quantitative examination of hibernacula microhabitat of many tortoise species.

In this study, we examined the microhabitat and thermal characteristics of hibernacula for a prairie-dependent

reptile, the ornate box turtle, using radio telemetry and temperature dataloggers. Our objectives were to 1) quantify the phenology of initiation and termination of turtle hibernation, 2) describe thermal environment of hibernacula and test whether the thermal environment of hibernacula differs significantly from ambient conditions for turtles during hibernation, and 3) examine whether soil and vegetation microhabitat variables of hibernacula differs significantly from random locations within home ranges of turtles.

Methods

We conducted our study within the Goose Lake Prairie Nature Preserve (GL; 1027 ha) and Wilmington Shrub Prairie Nature Preserve (WP; 58 ha)/Kankakee Sands Preserve (KSP; 225 ha) located in Grundy and Will Counties, IL, USA, respectively (Fig. 1). Both locations are characterized by mixed herbaceous prairie plants, marshes and secondary succession hardwood forest. Active management at each location is conducted in the form of prescribed burning, invasive species management and vegetation mowing. Both study sites were comprised of contiguous remnant and restored prairies adjacent to agricultural or forested landscapes (Fig. 1).

During April to August 2014 and May 2015 turtles were found using visual encounter surveys and trained turtle dogs. Once a turtle was captured, we measured morphological variables and affixed radio transmitters (model R1850 [12 g] or model R1680 [3.6 grams], Advanced Telemetry Systems) to a single carapacial scute using 5-min epoxy. We used ground-based radio telemetry to monitor turtles using a receiver (R410, Advanced Telemetry Systems) and a three-element yagi antenna between one to three times per week until hibernacula locations were established, and then one to three times per week beginning in March each year. For each radio-location, we recorded GPS coordinates using a handheld global positioning system unit (Garmin). During winter months during turtle dormancy, hibernacula locations were monitored closely for any indications of turtle movement in and out of the burrow (e.g. upturned, damp soil or sand). Telemetry activities were approved by the Loyola University Chicago Institutional Animal Care and Use Committee (Project 1405), including the use of radio-transmitters that equaled less than 5% of turtle body mass.

To measure turtle carapace temperature (T_c), we secured Thermochron iButton dataloggers (model DS1921G, Dallas Semiconductor) layered in black plastic coating to a single carapacial scute adjacent to the transmitter. Ambient ground temperature (T_g) data were derived from a coated iButton placed at ground level at each site ($n = 2$) each year. Each datalogger was programmed to record temperature every 240 min. Coating Thermochron iButton dataloggers has been found to have a measurable, yet small (0 to 1.3°C) influence on recorded temperature measurements (Roznik and Alford 2012). Thermal data (T_c and T_g) were categorized into hibernacula periods of 2014 (3 September 2014 to 5 May 2015), and 2015 (3 September 2015 to 10 May 2016). At the end of the 2014 hibernation period, iButtons were replaced on existing turtles and affixed to newly found turtles at both

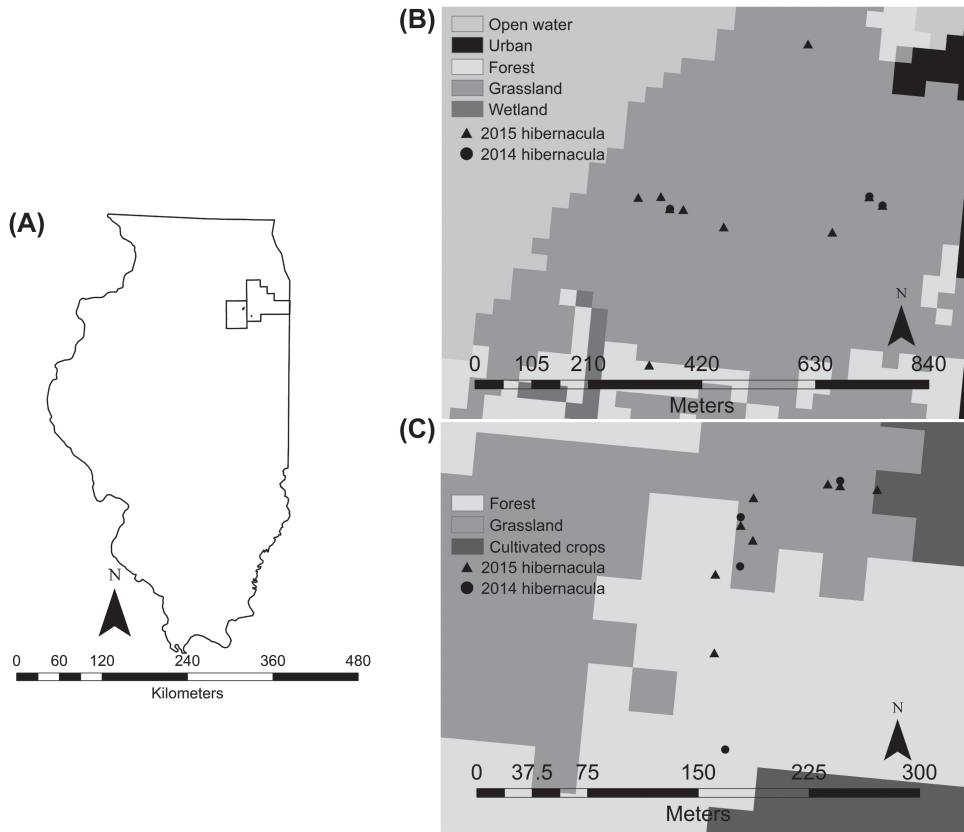


Figure 1. (A) Map of Illinois, Will and Grundy Counties, Illinois, and locations of WP/KSP (left) and GL (right) within Will and Grundy Counties, (B) map of GL and (C) WP/KSP with associated land use and 2014 and 2015 hibernacula locations.

sites. At the end of the 2015 hibernation period, iButtons were removed from all turtles at both sites. Turtle carapace temperatures reflect the turtle's immediate surrounding environment, and previous studies have found that this measure is similar to body temperature in small turtles (Grayson and Dorcas 2004, Shen et al. 2013). For five turtles, we identified hibernacula in both 2014 and 2015, four of which chose the exact same location for hibernacula each year. In 2014, turtle frequency 503 was lost from transmission until May 2015. However, iButton data was obtained at that time and analyzed for 2014. That turtle was refitted with a radio transmitter and iButton for 2015. In 2014, hibernacula data was recorded for turtle frequency 203, but following emergence the iButton was lost and therefore T_c data for 203 was not included in 2015 analysis. In 2015, the iButton for turtle frequencies 521, 741 and 801 were lost during termination resulting in lack of T_c data for those turtles.

We considered an animal to be hibernating when it did not move for at least 30 days after September of each year. We quantified abiotic and biotic variables at each hibernation plot within GL ($n=3$ total in 2014 and $n=10$ in 2015) and WP/KSP ($n=4$ total in 2014 and $n=8$ in 2015), and at an equal number of corresponding random points (selected using ArcMap) within the minimum convex polygon of all collected turtle points within a site (J. R. Milanovich pers. comm.) during each year. We estimated percent (%) composition of bare ground, leaf litter, coarse woody debris, herbaceous ground cover and shrub

cover within a one meter diameter of the center of each location at each hibernacula and random location in November of 2014 and 2015 (within one week of all hibernacula being identified, Table 1). Canopy cover was taken with a spherical densitometer at the center of each hibernacula or random plot by holding the densitometer approximately 1.2 m above ground level. Further, we extracted soil cores collected from each corner of the square PVC sampling unit using a pentar (2.5 cm diameter \times 25 cm length). Soil samples from each plot were combined, air dried and analyzed for pH, percentage (%) of organic matter, sand, silt and clay by the Kansas State University Soil Testing Laboratory.

Statistical analyses

To maintain statistical independence from the consequence of examining the same turtle hibernacula across two years, we used Mann–Whitney U -tests to examine the difference between 1) microhabitats and 2) soil variables between hibernation and random plots within 2014 and 2015 separately. We used paired t -tests to examine whether the thermal environment of hibernacula was significantly different than ambient temperatures by testing whether mean daily T_c temperature (six values per day) varied significantly between mean daily T_g during the hibernation period in 2014 and 2015. Standard (non-paired) logistic regression was used to compare turtle hibernacula with random plots within each year (to maintain independence). Eleven candidate models

were selected based on soil and vegetation microhabitat variables and were ranked using Akaike's information criterion corrected for small sample size (AIC_c). Final models (Table 2), with the greatest AIC weight (w_i) and the lowest AIC_c (and those within 2 AIC_c units) were considered the best supported. Odds ratios were calculated to assess the effect size of microhabitat variables selected for the final set of models.

Results

In 2014, box turtles at GL and WP/KSP initiated descent into hibernacula between 15 September to 3 October 2014 when ambient temperatures averaged between 16.7°C (± 1 SD = 0.8) and daylight averaged 12:07 h (± 1 SD = 14.4 min). In 2015, initiations occurred between 10 September to 9 October when ambient temperatures averaged 16.6°C (± 1 SD = 0.6) and daylight averaged 12:06 h (± 1 SD = 22.5 min) (Supplementary material Appendix 1 Table A1). The 2014 cohort of turtles terminated hibernation and ascended from hibernacula between 10 April to 5 May 2015 when T_g averaged 11.7°C (± 0.5 SD) and T_c averaged 14.8°C (± 1.4 SD) (Fig. 2A, Supplementary material Appendix 1 Table A1). The 2015 cohort of turtles ascended from hibernacula between 13 April to 10 May 2016 when T_g averaged 15.3°C (± 1.5 SD; Fig. 2B, Supplementary material Appendix 1 Table A1). In 2015, iButton error prevented measures of T_c during emergence,

so these values were not reported for many turtles. In 2014 and 2015, respectively, the time spent in hibernacula varied between 191–241 and 187–225 days (Supplementary material Appendix 2 Table A2). During the 2014 and 2015 hibernacula periods, there was 133 and 92 days at or below freezing at both sites (T_g), respectively. However, during hibernation no T_c recorded a temperature below 0.5°C (Fig. 2A–B). In addition to the similarity in initiation and termination dates across the two years, there were no significant differences in T_g within each site across years during the hibernacula initiation (Mann–Whitney U -test, $Z = 0.530$, $p = 0.598$). Mean daily T_c significantly varied from mean daily T_g during the hibernation period for all turtles (all p -values ≤ 0.05 ; Supplementary material Appendix 1 Table A1).

In 2014, there was significant variation among soil composition of % clay between hibernacula and random locations, but in 2015 the differences were significant for % clay, silt organic matter and sand (Fig. 3, Supplementary material Appendix 3 Table A3). In both cases hibernacula had lower % of clay, and in 2015 hibernacula had lower % silt and organic matter and higher percent sand compared to random locations. Microhabitat vegetation had lower % herbaceous ground cover compared to random locations in 2014 and higher % bare ground, leaf litter and shrub cover in 2015 (Table 1). Among the best-supported logistic regression

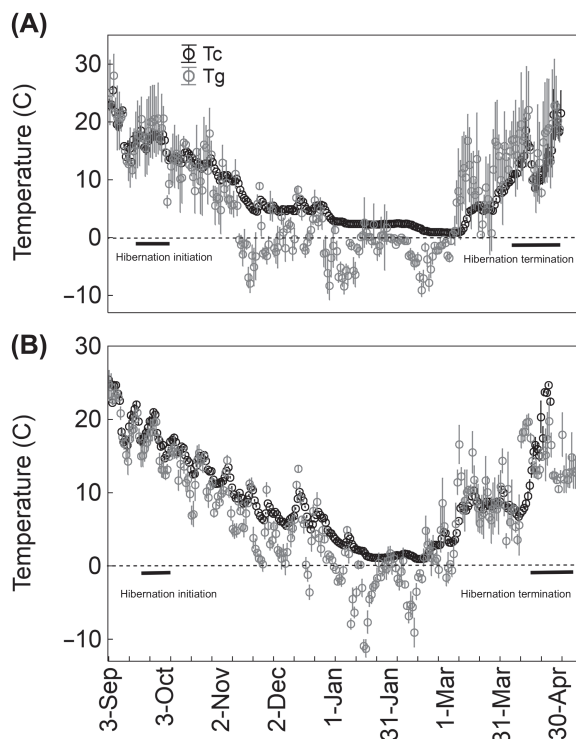


Figure 2. Daily mean temperature ($^\circ\text{C}$, SD) of T_c (turtle carapace; black circles and bars) and T_g (ambient ground; grey circles and bars) between (A) 3 September 2014 to 10 May 2015 and (B) 3 September 2015 to 10 May 2016 for turtles at GL and WP/KSP. Black horizontal bars indicate time periods of initiation into and termination out of hibernacula.

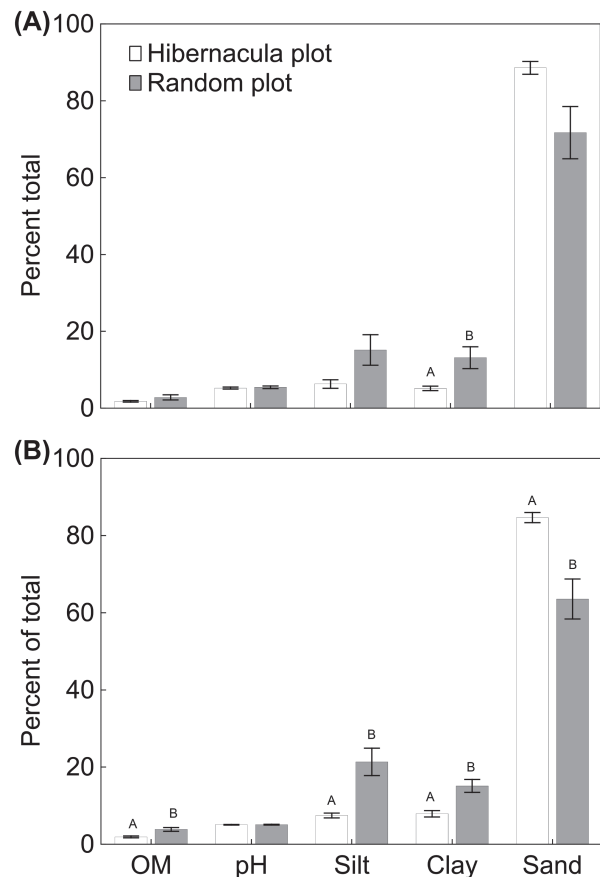


Figure 3. Mean (\pm SD) of soil characteristics of hibernacula and random plots for (A) seven box turtles within GL and WP/KSP in 2014, and (B) 18 box turtles within GL and WP/KSP in 2015. Different upper case letters represents statistical significance.

Table 1. Vegetation microhabitat variables (mean \pm SE) and significant Mann–Whitney *U*-test differences for hibernacula plots of ornate box turtles ($n = 18$) and random plots within the turtle home ranges ($n = 18$) across WP/KSP and GL during 2014 and 2015. Variables significantly different between hibernacula and non-hibernacula plots are in bold.

Microhabitat variables	Hibernacula (%)	Non-hibernacula (%)	Statistic	p
2014				
Bare ground	22.2 \pm 25.4	7.8 \pm 13.1	U = 16.0, Z = 0.307	0.318
Leaf litter	20.0 \pm 32.3	5.6 \pm 13.8	U = 11.0, Z = 1.661	0.097
Coarse woody debris	2.9 \pm 7.6	0.1 \pm 0.4	U = 24.0, Z = 0.000	0.710
Herbaceous ground cover	44.6 \pm 25.0	79.9 \pm 19.0	U = 7.0, Z = -2.172	0.013
Shrub cover	0.0	0.4 \pm 0.9	U = 59.5, Z = -0.831	0.209
Canopy cover	5.0 \pm 8.0	2.4 \pm 6.3	U = 46.0, Z = 0.767	0.383
2015				
Bare ground	16.4 \pm 22.4	3.5 \pm 6.6	U = 63.0, Z = 3.116	0.002
Leaf litter	26.6 \pm 28.7	14.2 \pm 22.7	U = 94.0, Z = 2.136	0.033
Coarse woody debris	3.2 \pm 4.9	14.2 \pm 30.0	U = 140.0, Z = 0.680	0.496
Herbaceous ground cover	41.5 \pm 34.8	55.8 \pm 33.3	U = 122.0, Z = -1.250	0.211
Shrub cover	12.0 \pm 16.9	1.0 \pm 0	U = 73.0, Z = 2.800	0.005
Canopy cover	1.9 \pm 3.1	1.3 \pm 5.3	U = 128.5, Z = 1.044	0.296

models (based on [AIC_c] values and associated diagnostics), sand, silt, clay (2014 and 2015) and herbaceous ground cover (2014) remained significant explanatory variables for predicting the selection of hibernacula (Table 2).

Discussion

Our results offer several key points that could help design or refine management plans for prairie ecosystems where box turtles are present. First, phenology of ornate box turtles in the northern part of their range is similar. In Illinois (our study), Wisconsin, Iowa and Nebraska, ornate box turtles initiate hibernation in late September to mid-October and terminate hibernation in April which equates to being in hibernacula between 166 to 216 days (Legler 1960, Metcalf and Metcalf 1970, Doroff and Keith 1990, Converse et al. 2002, Bernstein and Black 2005), which is similar to the range of days found in our study (Supplementary material Appendix 1 Table A1). Although we did not specifically test this hypothesis because we did not measure soil temperatures directly, our results visually corroborate Grobman (1990) that show ambient temperatures at ground level above 7°C several days may initiate termination from hibernacula (Fig. 2). We also found a number of turtles appeared to have ‘tested’ emergence or positioned themselves at the top of the burrows prior to actual emergence and many turtles in April were subsequently found partially buried at other locations.

Studying eastern box turtles, Currylow et al. (2013) suggested burrow depth was a factor contributing to hibernacula emergence, as turtles that were deeper in substrate emerged later in the season.

Models of poikilothermic cold tolerance suggests a strategy that permits freezing (i.e. freeze-tolerance) is advantageous compared to freeze-avoidance (Voituron et al. 2002). Adult eastern box turtles *Terrapene carolina* have freeze tolerance capabilities that permit up to 58% of their body to freeze (Costanzo and Claussen 1990) and this trait extends to ornate box turtles, which can withstand consistent freezing temperatures (Costanzo et al. 1995). In this study, *Tg* recorded an average of 112 days below freezing across two years, and other studies found box turtles in hibernacula are frequently exposed to below freezing temperatures for several days (Bernstein and Black 2005, Currylow et al. 2013). However, we found significant differences in *Tc* and *Tg* during the hibernation period (Fig. 2, Supplementary material Appendix 1 Table A1) and did not record a temperature below freezing from a turtle, which was similar to other studies showing the majority of box turtles in hibernacula do not experience sub-zero temperatures (Doroff and Keith 1990, Claussen et al. 1991, Bernstein and Black 2005, Currylow et al. 2013). Further, we found no evidence of temporary movement up to and along the ground surface during the winter months, based on highly stable *Tc* measurements (Fig. 2) and regular close inspection of hibernacula burrows. This suggests turtles may be actively attempting

Table 2. The best-fit logistic regression models for turtle hibernacula, as determined by AIC_c.

Microhabitat variables	AIC _c	wi	Estimate \pm SE	Odds ratio
2014				
Herbaceous ground cover	17.667	0.288	0.065 \pm 0.03	1.067
Clay	17.684	0.285	0.365 \pm 0.259	1.441
Sand	18.976	0.150	-0.121 \pm 0.075	0.886
Silt	19.911	0.094	0.181 \pm 0.111	1.198
2015				
Sand	38.698	0.409	-0.124 \pm 0.050	0.883
Silt	38.890	0.372	0.201 \pm 0.088	1.222
Clay	40.571	0.160	0.273 \pm 0.100	1.314

to avoid freezing temperatures in addition to implementing freeze tolerance strategies.

Our findings indicate that ornate box turtles select winter hibernacula with soil microhabitats that contain a higher percentage of sand and lower percentages of clay and silt. Above-ground preferences are driven by lower herbaceous ground cover, but we also found higher percentages of bare ground or leaf litter associated with hibernacula (Fig. 2, Supplementary material Appendix 1 Table A1). Although the sample size of 2014 hibernacula plots was small ($n = 7$), our analysis suggests similar microhabitat variables influence location of hibernacula across 2014 and 2015. The preference for sandy soils with less herbaceous ground cover is likely a result of ease of burrowing. Burrowing may be a necessary adaptation for turtles to survive long-term below the frost line. However, the selection of sandy soils is counterintuitive when one considers these soils retain relatively little insulation against freezing temperatures. For example, in painted turtles *Chrysemys picta*, resistance to freezing is greatly improved in sandy soils when clay or organic matter is added (Packard and Packard 1997, Costanzo et al. 1998). It appears that box turtles may select for soils that provide less resistance to burrowing compared to soils that may have components to assist in freeze prevention (e.g. a higher percentage of organic matter). Although we hypothesize sandy soils may not provide much freeze resistance, these sites were presumably buffered enough that none of the monitored turtles experienced freezing temperatures during hibernation.

Prairie management practices are typically widespread in use and acreage and can have measurable impact on vertebrate species by altering habitat or initiating risk of injury or death (e.g. from prescribed burning) if conducted within sensitive habitats or across sensitive time periods (Clark and Kaufman 1990, Johnson 1997, Coppedge et al. 2008, Larson 2014). The life history of species in northern latitudes, especially poikilotherms, is largely dictated by thermal tolerance where species must adapt to large scale temperature fluctuations (Sunday et al. 2011). Prairie-adapted vertebrates use a variety of habitat types seasonally for thermoregulation, reproduction, nesting and dormancy purposes (Knopf and Samson 2013). Management practices must follow these species requirements to reduce the risk of altering habitat during sensitive time periods (e.g. initiation/termination from hibernation, reproduction). This current study offers key insights into the phenology, thermal environment and microhabitat selection of box turtle hibernacula. Understanding the phenology of prairie-adapted species has led to the refinement of prairie prescribed burning management practices for plants (Pavlovic et al. 2011, Towne and Craine 2014), insects (Cook and Holt 2006) and reptiles (Gardiner et al. 2013). Based on our findings, we suggest that prescribed burns conducted in the spring season occur before 10 April to prevent above ground mortality of box turtles. Furthermore, we suggest managers strive to maintain sandy areas with leaf litter and low shrub cover to maximize suitable habitat for hibernacula for box turtles. Lastly, as global change (climate and land-use) continues to shape ecological communities, there is increased importance to maintain suitable habitat and gain a better understanding on phenology

and thermoregulation requirements for sensitive species. Known and predicted impacts of global climate change on herpetofauna can impact phenology of species (Blaustein et al. 2010), and impacts to thermoregulation could impact reproductive rates and evolution (Medina et al. 2012).

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Supplementary material (available online as Appendix wlb-00295 at <wildlifebiology.org/appendix/wlb-00295>). Appendix 1–3.