

Effects of Prairie Degradation and Restoration on Box Turtle Thermal Ecology

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ABSTRACT.—Prairie habitat loss in the United States has led to population declines in many prairie-associated species, including Ornate Box Turtles (*Terrapene ornata*). Northwest Arkansas is an intergrade zone between the prairie-dwelling *T. ornata* and the more forest-associated Three-Toed Box Turtle (*Terrapene carolina*). As such, limited information exists on the potential differences in physiology and thermal ecology between the two box turtle species and how those differences might influence their habitat use. We addressed gaps in our knowledge of the thermal and spatial ecology of *T. ornata* and *T. carolina* with a three-part study. First, we compared the thermal profiles of refugia, open, and vegetated microhabitats across degraded prairie, restored prairie, and adjacent forest macrohabitats using operative temperature models and a linear mixed effect model. Second, we measured total evaporative water loss of both species across a range of body sizes. Finally, we fitted a subset of turtles with iButton data loggers and monitored them in the field to examine carapace temperatures and habitat use. Operative temperature models recorded high, largely homogeneous temperatures across microhabitats in degraded prairie and heterogeneous temperatures across restored prairie microhabitats, while forest habitat maintained stable, cool temperatures. Both species exhibited similar evaporative water loss rates; however, *T. ornata* experienced a broader range of temperatures in the field. *Terrapene ornata* were exclusively found in prairie habitat, whereas *T. carolina* was often found in forested habitats and subsurface refugia. Our results demonstrate key differences in box turtle thermal biology and highlight suboptimal thermal characteristics in degraded prairie and forest habitat that should be considered in prairie restoration and management for *T. ornata* conservation.

Less than three percent of historic prairie remains in the United States, supporting endemic species of conservation concern from a wide range of taxa (Sampson and Knopf, 1994; Refsnider et al., 2012). Prairies that have been altered for human use can become inhospitable for prairie-associated species unless they are actively managed to preserve ecosystem structure and function (Sampson and Knopf, 1994). Prairie loss and habitat degradation via changes in vegetation composition (e.g., canopy encroachment) are thought to be primary reasons for the decline of many reptile and amphibian species, such as the Crawfish Frog (*Lithobates areolatus*; Redmer and Parris, 2005). Changes in thermal conditions caused by increased canopy cover and altered vegetation structure likely alter thermoregulatory opportunities and activity time for reptiles (Grant and Dunham, 1988; Brewster et al., 2018), ultimately affecting vital rates and population dynamics.

Arkansas is in the transition zone from tallgrass prairie to oak-hickory forest and is where many prairie-associated species reach their eastern range limits. Habitat loss and fragmentation of the remaining small patches of remnant prairie by roads, agriculture, and urban development in this region restrict populations of prairie-associated wildlife, including ornate box turtles (*Terrapene ornata*) (Brye et al., 2004; Refsnider et al., 2012). *Terrapene ornata* is declining in eastern portions of its range (Refsnider et al., 2012), including in Arkansas, where it is listed as a Species of Greatest Conservation Need by the Arkansas Game and Fish Commission (AGFC, 2015). The degradation of former prairie for agriculture and the encroachment of woody plants because of fire suppression and loss of large grazing mammals likely decreases habitat quality for *T. ornata* and may facilitate colonization of former prairies by generalist species such as Three-Toed Box Turtles (*Terrapene carolina*). Habitat

structure influences *T. ornata* and *T. carolina* distribution, as *T. ornata* requires open-canopy conditions to maintain preferred body temperatures, which are higher than those preferred by *T. carolina* (Legler, 1960; do Amaral et al., 2002). *Terrapene ornata* are more effective behavioral thermoregulators at higher temperatures and are primarily found in grasslands, from semidesert to tallgrass prairie when compared to *T. carolina* that are found primarily in forested habitats (Reagan, 1974; Sturbaum and Riedesel, 1974). *Terrapene ornata* and *T. carolina* occur in sympatry within a narrow portion of their respective ranges. In regions where both species occur, *T. carolina* are often more common, which might be related to habitat degradation and fragmentation of prairies and other open-canopy habitats (Grant and Dunham, 1988; Converse and Savidge, 2003). Additionally, *T. ornata* thermal preferences might indicate a physiological difference, such as resistance to water loss, which allows the species to occur in more-arid, open-canopy habitats.

Our study aimed to: 1) compare thermal conditions in restored prairie, degraded prairie, and forested habitats; 2) evaluate differences in total evaporative water loss (TEWL) rates between *T. ornata* and *T. carolina*; and 3) characterize and compare thermal conditions experienced by each species. To address these objectives, we developed thermal profiles for degraded prairie, restored prairie, and forested macrohabitats in Northwest Arkansas using operative temperature models. Within each macrohabitat, we designated shaded, open, and refugia microhabitats. We hypothesized that degraded prairie would have the highest average temperatures, followed by restored prairie microhabitats, with forested areas exhibiting the lowest average temperatures across all microhabitat types. We also conducted lab trials to compare TEWL of *T. ornata* and *T. carolina*. We hypothesized that *T. ornata* would be more resistant to desiccation and would exhibit lower rates of TEWL. Following TEWL trials, we returned turtles to their original locations fitted with iButton temperature loggers and radio

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DOI: 10.1607/22-016

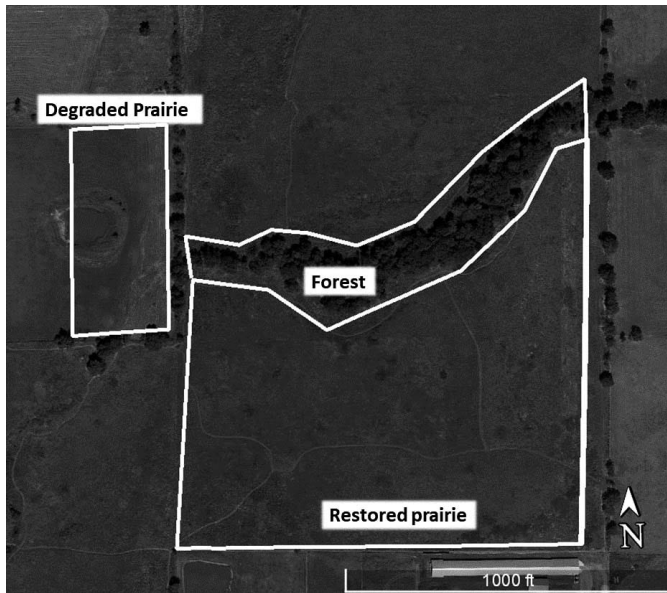


FIG. 1. Chesney Prairie site (forest and restored prairie) and neighboring agricultural fields (degraded prairie) in Arkansas.

transmitters to monitor their habitat use and track carapace temperatures. We hypothesized that *T. ornata* would exhibit higher carapace temperatures and utilize restored prairie microhabitats more often than would *T. carolina*.

MATERIALS AND METHODS

Study Sites.—Chesney and Stump Prairies are fire-maintained, restored, and remnant prairies in Benton County, Arkansas and are 33.59 ha and 7.40 ha in size, respectively. Both Chesney and Stump Prairies feature diverse native plant communities and have retained prairie mounds, the minor topographical features typical of intact prairie habitat in this region (Durre et al., 2019). Chesney Prairie contains an ephemeral stream that forms a wooded riparian corridor through native tallgrass prairie vegetation (Fig. 1). An agricultural field bordering Chesney Prairie represented degraded prairie, characterized by a monoculture of tall fescue grass (*Lolium arundinacea*) and lacking the prairie mounds present in many remnant or restored prairies, likely because of past plowing.

Thermal Characteristics of Forest, Restored, and Degraded Prairie Habitats.—We used operative temperature models to record the thermal conditions available to box turtles within selected microhabitats at Chesney Prairie (Fig. 1). We constructed models following the design developed by Radzio and O'Connor (2017), using 414 mL, 7.62-cm diameter × 11.27-cm high metal cans painted with Rust-Oleum Painter's Touch 2X Flat Gray Primer General Purpose Spray Paint. We attached a 3.5-cm plastic spacer and Thermochron iButton temperature data logger (Maxim Integrated DS1921G-F5) to the bottom of each sealed, horizontal can and each model was tethered in place in its microhabitat.

We placed 30 models in the restored area of Chesney Prairie, 30 models in the forested riparian area of Chesney Prairie along Sager Creek, and 30 models in the fescue monoculture fields representing degraded prairie. Within both restored prairie and forested habitat, we placed 10 models in subterranean refugia, 10 models in open, low-vegetation areas, and 10 models in areas shaded by vegetation. We characterized refugia microhabitat as any underground shelter large enough for use by an adult box

turtle (primarily mammal burrows), open microhabitat as a 1-m² area with vegetation <1 m high with some bare soil groundcover and no vegetation cover directly over model locations, and shaded vegetation microhabitat as a 1-m² area with no bare soil cover, dense vegetation, and vegetation cover directly over model locations. Because the degraded prairie site lacked the subterranean refugia found in the forested and restored prairie sites, we placed 10 models in open areas, 10 models in areas shaded by vegetation, and an additional 10 models in the thickest clumps of vegetation (hereafter referred to as refugia/thick vegetation) to represent the most sheltered microhabitat that was available to turtles. We selected microhabitat locations by walking transects through each macrohabitat until finding a microhabitat site that met the a priori specifications for each microhabitat type. We placed models at least 3 m from any previously set model. We left all models in the field for 9 days beginning 10 July 2019 and programmed iButtons to record model temperature every 30 min. After the sampling period, we collected models and downloaded the data. During the sampling period, four models from each site were either lost or malfunctioned. The macrohabitat sample sizes remained even, although microhabitat samples became slightly unbalanced among macrohabitats.

Total Evaporative Water Loss.—We measured rates of TEWL for *T. carolina* and *T. ornata* following the general methods of Sturbaum and Riedesel (1974) and Sturbaum (1981). We collected 6 *T. ornata* and 11 *T. carolina* from the field, with masses ranging from 66 g to 386 g. We primarily collected turtles at Chesney Prairie, but one *T. ornata* and two *T. carolina* were collected at Stump Prairie and one *T. ornata* was collected at Cherokee Prairie, a nearby protected prairie remnant in Franklin County, Arkansas. We collected turtles in June and July 2019 by searching tallgrass prairie and mowed trails on foot, 0700–1200 h.

We maintained all turtles in 75-L aquaria with turtles from the same capture location for a minimum of 7 days at room temperature (approximately 22°C), with access to water ad libitum, to ensure that individuals were postabsorptive and fully hydrated. Following the acclimation period, we placed each turtle in a small hardware cloth cage. Cage dimensions varied according to individual turtle size and allowed enough room for each turtle to pivot unobstructed but did not allow for excessive movement. We allowed each turtle a 24-h acclimation period within the cage at room temperature. Following acclimation, we placed each turtle in an environmental chamber at 27°C and 50% relative humidity for 7 days with a plastic tray under each cage to monitor for feces and urine. We weighed each turtle and its respective cage upon placement in the environmental chamber and every 24 h thereafter. We ended trials after 8 days or when greater than 10% body weight was lost.

Carapace Temperatures of Free-Ranging Turtles.—Following TEWL trials, we fed and rehydrated all turtles for at least 1 wk before release at their original capture locations. We fitted four *T. ornata* and four *T. carolina* from Stump and Chesney Prairies with radio transmitters (Holohill Model RI-2B) and iButtons fixed to the carapace with JB Weld Waterweld Epoxy. The additions accounted for <10% of the body weight of each turtle to ensure that activity was not inhibited. We tracked turtles two times per week following release and noted exact coordinates and macrohabitat–microhabitat type based on the same habitat categories used for operative temperature models. We tracked turtles five times for a total of 20 *T. carolina* and 19 *T. ornata* observations after

one transmitter malfunction. After 9 days we removed the iButtons, and after 3 wk we removed the transmitters.

Data Analyses.—Using program R (v.4.1.2) (R Development Core Team, 2021) package *lme4* (Bates et al., 2015) and linear mixed models (LMMs), we compared thermal conditions of the different macro- and microhabitats at four different time points each day (0800 h, 1200 h, 1600 h, and 2000 h). The time points used encompassed the reported daily activity windows of both species of interest, including morning emergence and evening retreat to refugia (do Amaral et al., 2002). We treated macrohabitat and microhabitat as fixed effects of interest and individual thermal model ID (treated as the experimental unit) and day as random effects (Harrison et al., 2018). To account for diel differences in thermal conditions, we ran separate mixed models for each time point comparing macro- and microhabitats over the 9-day sampling period. We used this analysis to detect effects of macrohabitat type, microhabitat type, and their interactive effects on mean model temperature at multiple daily time points during the sampling period (e.g., mean temperature of all restored prairie shaded vegetation models at 0800 h, 1200 h, 1600 h, and 2000 h across all sampling days). The resulting model structure incorporating both fixed and random effects for each of the four time points, t , was: $\text{Temp}_t = \text{MacroHab} + \text{MicroHab} + \text{MacroHab} * \text{MicroHab} + (1|\text{ModID}) + (1|\text{Day}) + \epsilon_{\text{error}}$ where the $1|$ terms denote the application of random intercepts to the random effects in the model. We compared the fit of these full models against simpler models to find the most parsimonious models for each time point (Burnham and Anderson, 2004) and found the full interaction models to be the best fit by Akaike information criterion ($\Delta\text{AIC}_c > 2$) for all time points. We tested significance of fixed effects and interactions in each model using the Type II Wald chi-square test through the analysis of variance (ANOVA) function in R package *car* (Fox and Weisberg, 2019). Interaction effects were significant in each time point; thus, we focused our interpretation on interaction effects rather than main effects.

In R package *stats*, we used an analysis of covariance (ANCOVA) to compare the average TEWL per 24 h between species with initial mass as a covariate (Rutherford, 2011). Additionally, because of our small sample sizes, we performed a power analysis using the 'ANCOVA_analytic' function using our TEWL effect size data within the superpower package (Lakens and Caldwell, 2021) to determine the sample size needed to detect a true difference with 80% power. To compare carapace temperatures of free-ranging turtles by species, we used three independent t -tests to compare the average daytime carapace temperature, average daily maximum carapace temperature, and average daily minimum carapace temperature between species with the *stats* package in R, with a Bonferroni-adjusted significance level set at $\alpha = 0.017$ to account for multiple temperature comparisons using the same turtles. All analyses were completed using program R v. 4.1.0 (R Core Team, 2021).

RESULTS

Prairie Thermal Profiles.—Forest, restored prairie, and degraded prairie had average maximum daily temperatures of approximately 29, 32 and 40.5°C, respectively. The interaction of macrohabitat type and microhabitat type was significant at each time point (all $P < 0.05$), with forested and degraded prairie macrohabitat exhibiting relatively little temperature variation among microhabitats throughout the day (<7°C) and restored

prairie featuring greater heterogeneity in available temperatures among microhabitat types (<18°C; Fig. 2). Forest habitat remained cooler during the day and warmer at night, while degraded prairie had the widest temperature fluctuations, with the highest daytime and lowest nighttime temperatures of the three macrohabitats (Fig. 3). Models in open degraded prairie and shaded degraded prairie microhabitats experienced the highest day (>45°C) and lowest night temperatures (<15°C) (Fig. 3). Open restored prairie had an average daily maximum temperature of over 55°C. However, shaded restored prairie temperatures only reached a maximum of 30°C and restored prairie refugia had an average daily maximum of approximately 25°C (Fig. 3).

Total Evaporative Water Loss.—The average rate of water loss for *T. ornata* was lower (range = 1.5 to 3.1 g/day, mean = 2.33) than *T. carolina* (range = 1.96 g/day to 4.94 g/day, mean = 3.24 g/day). After accounting for differences in body size with ANCOVA, rate of mass loss was not significantly different between species ($F_{1,14} = 1.2$, $P = 0.2$) and rate of loss increased with increasing body mass ($F_{1,14} = 23.3$, $P < 0.001$; Fig. 4). Power analysis suggested a total sample size of 44 is needed to detect a difference in TEWL rates between the species with 80% power using an $r^2 > 0.75$ and each species' effect size estimates.

Carapace Temperatures of Free-Ranging Turtles.—There were no significant differences in average daytime temperature, average daily maximum temperature, and average daily minimum temperature between species (Daytime Temperature: $t_6 = -2.36$, $P = 0.056$; Daily Maximum Temperature: $t_6 = 2.22$, $P = 0.068$; Daily Minimum Temperature: $t_6 = 1.67$, $P = 0.15$). *Terrapene ornata* average daily maximum temperature was 1.67°C higher than *T. carolina*, and *T. ornata* had an average daily low that was 0.5°C lower than *T. carolina* (Fig. 5).

Terrapene carolina were located under canopy cover 50% of the time, in shaded prairie 50% of the time, and never observed in the open (Fig. 6). *Terrapene ornata* were found in the open 16% of the time, in shaded prairie 84% of the time, and never under canopy cover. Notably, *T. ornata* were located on prairie mounds twice and within 1.5 m of an open trail edge five times. One *T. carolina* was found approximately 0.25 m into a burrow, and another was found buried in a form (subterranean shelter) with only its head and upper carapace exposed.

DISCUSSION

Our results provide important insight into the physiologies and thermal ecologies of *T. ornata* and *T. carolina* and the importance of thermal heterogeneity in prairie habitat to these species. Previous studies have documented habitat preferences and preferred body temperatures of these species in their core geographic ranges (Cureton et al., 2011). We identified differences in macrohabitat and microhabitat thermal characteristics among restored prairie, degraded prairie, and forested areas, and we show microhabitat selection patterns that might affect the ability of *T. ornata* to survive and reproduce in eastern portions of its range. Selection of warmer, more-open microhabitat by *T. ornata* support their documented preference for, and adaptation to, open canopy habitat. Operative temperature models demonstrated that restored prairie habitat provided considerably more thermal heterogeneity compared to degraded prairie and forest habitat. These findings highlight the importance of restoring and actively managing prairie habitat to limit woody plant encroachment and increase herbaceous plant

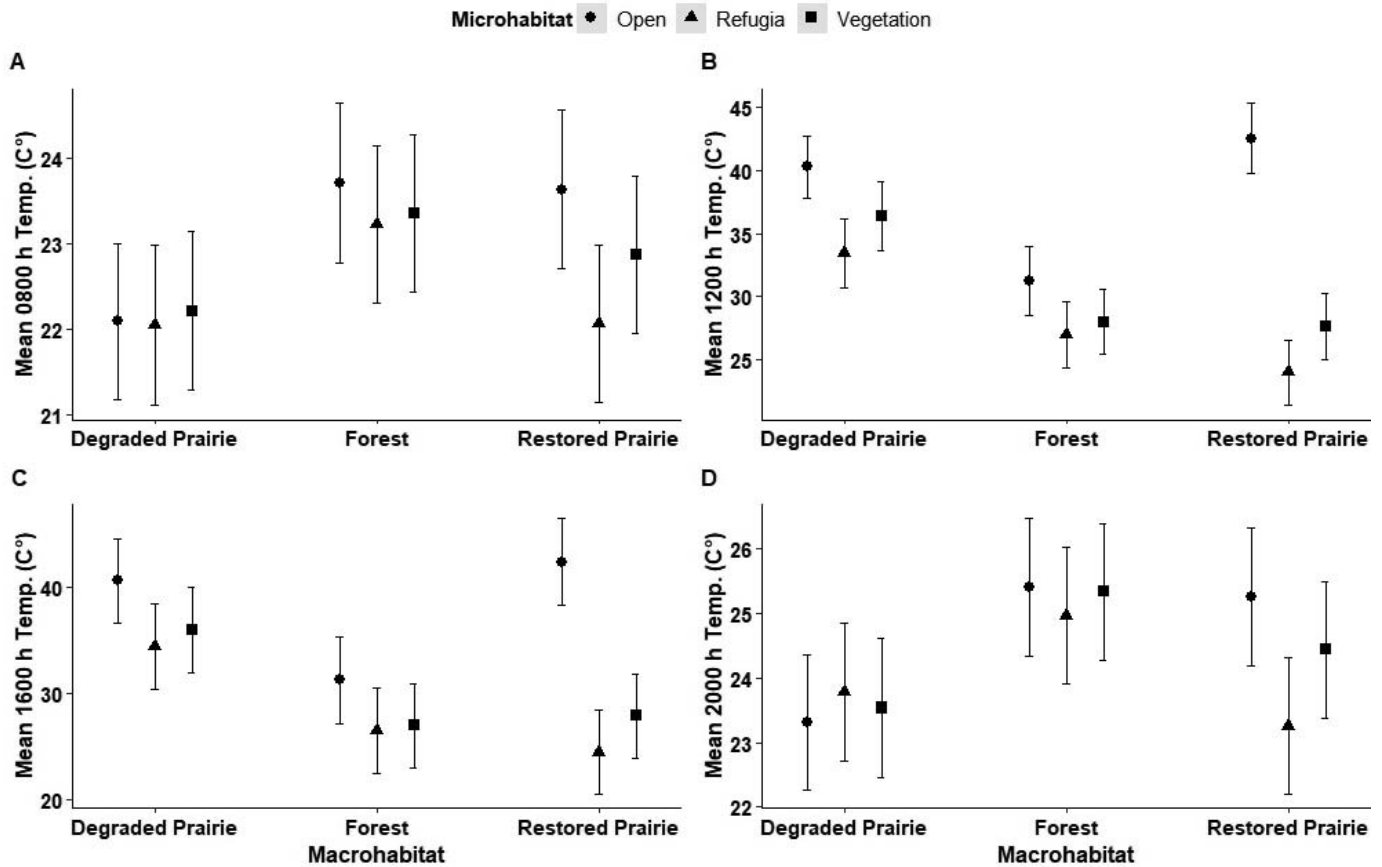


FIG. 2. Interaction plots showing mean operative temperature model temperatures at A) 0800 h, B) 1200 h, C) 1600 h, and D) 2000 h with 95% confidence intervals. Open microhabitat was a 1-m² area with vegetation <1 m high with some bare soil groundcover and no vegetation cover. Refugia microhabitat was any underground shelter large enough for use by an adult box turtle; for the degraded prairie, refugia references areas of thick vegetation because of a lack of refugia (see Methods for more information). Vegetation microhabitat was a 1-m² area with dense vegetation and vegetation cover, with no bare soil.

diversity and structural heterogeneity as an essential part of management plans for *T. ornata*.

When considering habitat thermal profiles, the macrohabitat-microhabitat interaction clearly highlights the differences in the range of temperatures available within each macrohabitat, driven by variation among microhabitat types. In degraded prairie and forest habitats, temperatures were similar among microhabitats, regardless of time of day. Although open restored prairie habitat (42.5°C) was warmer than open degraded habitat (40.3°C) at 1200 h, restored prairie refugia (24.0°C) and vegetation (27.6°C) microhabitats offered much cooler temperatures (Fig. 2). Refugia/thick vegetation (33.5°C) and vegetation (36.4°C) microhabitats within degraded prairie were both considerably warmer than the same microhabitat types in restored prairie and forested habitats at 1200 h. This pattern of high overall temperatures in degraded habitat and heterogeneous temperatures in restored prairie habitat was consistent across all four time points, while forested habitat remained both homogenous and at moderate temperatures throughout the day. Open microhabitat in restored prairie remained notably warmer than other restored prairie microhabitats throughout all time points, while refugia microhabitat in restored prairie was consistently among the coolest microhabitats among all macrohabitat-microhabitat combinations. Open habitat was also the warmest microhabitat type in degraded and forested macrohabitats but did not differ significantly from refugia and

vegetation microhabitats, even during the middle of the afternoon.

Preferred body temperatures of *T. ornata* (29°C) and *T. carolina* (27°C), as determined by lab trials in do Amaral et al. (2002), are well below any of the operative temperatures measured in degraded habitat during the 1200 h and 1600 h time points (Legler, 1960). This suggests that thermal conditions available in degraded prairie severely restrict the daily available activity windows of these species, leading to decreases in foraging opportunities, mate searching, and breeding activities (Grant and Dunham, 1988; Robertson et al., 2021). Although degraded prairie habitat structure is superficially similar to restored prairie habitat, degraded habitat does not provide the thermal heterogeneity among microhabitats necessary for box turtles to thermoregulate efficiently or shelter from extreme temperatures. The known critical thermal maximum (body temperature at which individuals lose their righting response) for *T. carolina* (39°C) is far below many of the operative temperatures recorded in degraded habitat, and both species could be at risk of direct mortality in degraded habitat during the hottest parts of the year. Although this study was completed during the hottest part of the year and likely captured some extremes, it was still within the active season for both species.

The forested habitat did not present a threat of direct mortality from overheating for either species, but the lower peak temperatures and minimal thermal gradient among microhabitats found in the forested site might result in less-

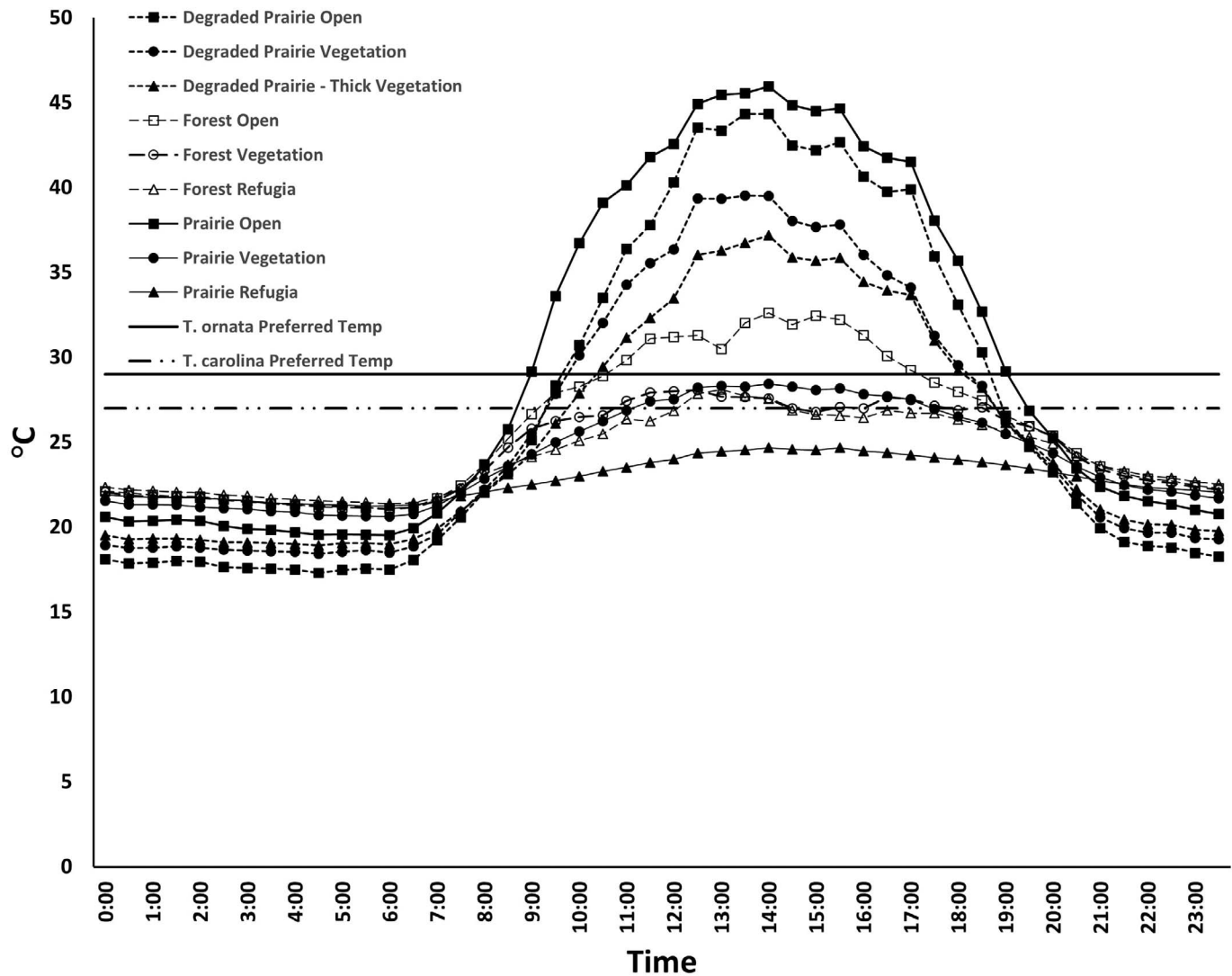


FIG. 3. Average temperatures of operative temperature models in refugia, open, and vegetation microhabitats within restored prairie, degraded prairie, and forest macrohabitats over a 9-day period beginning 10 July 2019, with lines representing laboratory-determined preferred body temperatures of *T. ornata* (29°C) and *T. carolina* (27°C) (do Amaral et al., 2002).

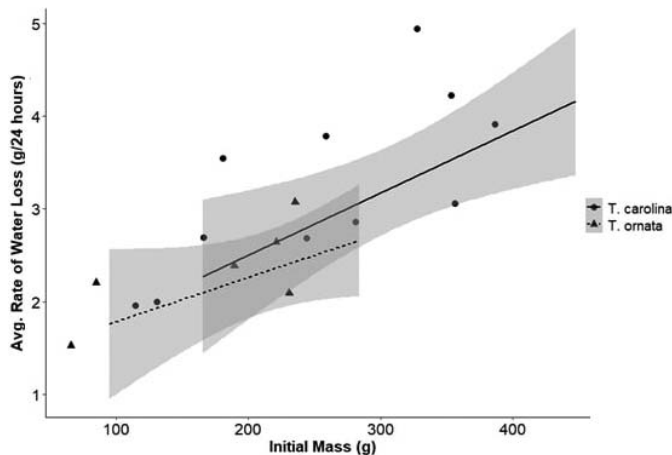


FIG. 4. Rate of total evaporative water loss of *T. carolina* ($n = 11$; slope = 0.008) and *T. ornata* ($n = 6$; slope = 0.004) measured during laboratory trials. Shaded areas represent 95% confidence intervals from ANCOVA.

efficient thermoregulation by requiring longer basking times to reach preferred body temperatures. Previous research has shown that even subtle changes to a population's thermoregulatory opportunities because of canopy encroachment can trigger a cascading effect, potentially leading to population declines and local extirpation (Brewster et al., 2018).

Small changes in temperature can negatively affect physiological processes in ectotherms. In many reptiles, digestive efficiency, passage time, and growth rates can be negatively affected by reductions in temperature, eventually influencing population vital rates (Sadeghayobi et al., 2011; Beaupre and Zaidan, 2012; Brewster et al., 2018). Over time, canopy encroachment can lead to local extinctions in reptile species with high preferred body temperatures that are dependent on prairie or open glade habitat (Webb et al., 2005). This might affect *T. ornata*, a prairie specialist, far more than *T. carolina*, a generalist species that can reach high densities in forested habitat. This type of habitat degradation should be an important consideration in *T. ornata* conservation. Higher average temperatures in degraded prairie, lower average temperatures in forest, and lack of microhabitat heterogeneity that leads to more

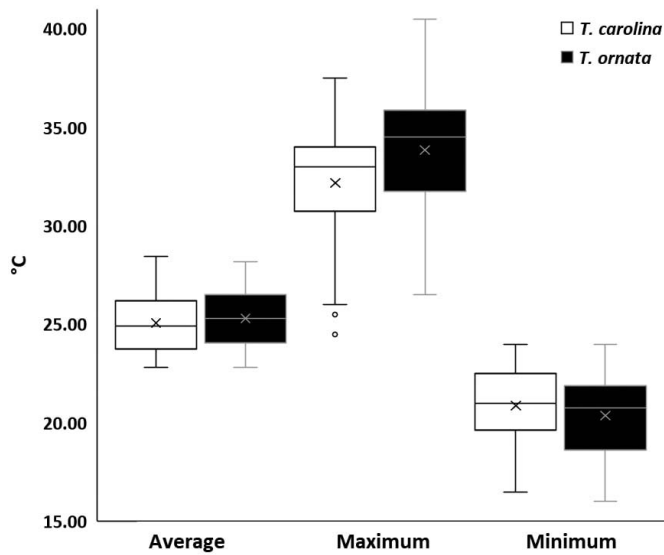


FIG. 5. Average daytime (0900–1800 h) carapace temperatures and average daily maximum and minimum carapace temperatures of live *T. ornata* ($n = 4$) and *T. carolina* ($n = 4$) over a 9-day tracking period. Medians are shown by horizontal lines inside boxes, means by $\times$, the 75th and 25th quartiles by tops and bottoms of boxes, respectively, the farthest points not exceeding 1.5 times the distance between quartiles by vertical lines and outliers by dots beyond vertical lines.

uniform temperature profiles in both habitat types might all have negative impacts on *T. ornata* populations.

In free-ranging turtles, differences in average daytime carapace temperature, average daily maximum carapace temperature, and average daily minimum carapace temperature between species were not statistically significant. While average daytime carapace temperature of *T. ornata* was 1.07°C higher than *T. carolina*, as predicted by lab thermoregulation trials (do Amaral et al., 2002), insights to be gained solely from carapace temperatures are limited in scope, and further research is needed to compare field body temperatures in known microhabitats to adequately understand how the thermal ecologies of *T. carolina* and *T. ornata* might differ in shared habitat. Comparatively, *T. carolina* tended to use more forest and refugia habitat while *T. ornata* tended to use more open restored prairie habitat. The microhabitat use we observed reinforces present knowledge on *T. carolina* preference for mesic, forested habitat and *T. ornata* preference for arid, open habitat (Converse and Savidge, 2003; Cureton et al., 2011). Bimodal activity patterns in both species and higher preferred temperatures in *T. ornata* highlight potential differences in the length of available activity time between the two species, depending on habitat thermal characteristics (Converse and Savidge, 2003).

Our TEWL results showed no statistically significant difference in water loss rates between the two species. This finding suggests that *T. ornata* may deal with the water balance demands of hot, arid environments through behavioral rather than physiological or morphological adaptations. DeNardo et al. (2004) suggested that Gila Monsters (*Heloderma suspectum*) use high cloacal evaporative weight loss (EWL) rates to facilitate evaporative cooling and extend activity periods in times of extreme heat. A similar mechanism could allow *T. ornata* to remain active during periods of extreme summer heat in prairies if they do not face challenges related to water limitation. Because our sample size was small and the limited availability of overlapping body sizes between species complicated these

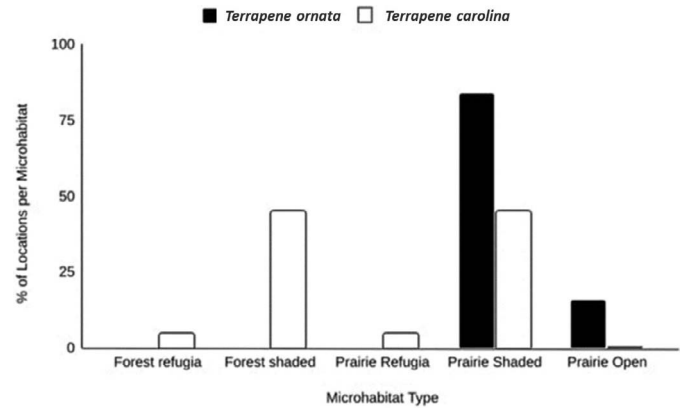


FIG. 6. Microhabitat use of radio tracked *T. ornata* ($n = 4$) and *T. carolina* ($n = 4$) in two small, restored prairies in Northwest Arkansas. Bars represent the percent of total observations located in each microhabitat type.

analyses, we completed a power analysis to identify a recommended sample size. Our results suggest higher sample sizes ($n_1 = 22$; $n_2 = 22$) are needed to confidently determine whether TEWL rates are significantly different between species. Further, increased sample size of similar-sized turtles, collection of individuals from throughout each species' range, and EWL trials conducted under a range of temperatures would further clarify EWL patterns of *T. ornata* and *T. carolina*.

Arid conditions may have historically excluded *T. carolina* from unfragmented prairies. Our observations of selection for more-open, warmer microhabitats by *T. ornata* support past observations of behavioral differences between the species (Stone and Moll, 2009). However, canopy cover intrusion and inadequate prescribed burning could allow *T. carolina* to invade landscapes that would otherwise be unsuitable for this species (Reagan, 1974; Robertson et al., 2021). Mesic conditions in degraded prairie with woody plant encroachment might allow for increased competition and hybridization between *T. carolina* and *T. ornata* (Cureton et al., 2011).

Results from habitat thermal profiles, carapace temperatures, and habitat use patterns combine to suggest that degraded prairies with altered vegetation and microtopographic characteristics provide suboptimal thermal conditions for *T. ornata*. Both agricultural areas and areas with encroaching canopy cover represent degraded habitats with limited thermoregulatory opportunities for *T. ornata*. Human encroachment and fire suppression have increased the amount of forested habitat in Arkansas' historic prairies and fragmented the few remaining remnant prairies. Forested areas, being more mesic and cooler than prairie, are more suitable for *T. carolina*, which has a lower preferred body temperature. The interspecific differences in thermal biology and behavior investigated in this study could help explain *T. carolina* prevalence, range expansion, and hybridization with *T. ornata*. Higher microhabitat temperature preferences highlight the importance of active management that maintains both open canopy and suitable refugia for remaining *T. ornata* populations in Arkansas. With limited remnant prairie remaining, restoration of degraded prairie and the use of management practices such as prescribed burning that limit canopy cover, remove invasive species, and preserve diverse microhabitats, are needed to provide optimal thermal conditions for *T. ornata*. Continued research into thermal preferences, microhabitat use across active seasons, and interspecific competition is needed to better understand the habitat

requirements and population dynamics of *T. ornata* and *T. carolina* in changing landscapes.

Acknowledgments.—We completed this research using capture and handling protocols reviewed and approved by the University of Arkansas Institutional Animal Care and Review Committee (AUP 19101) and Arkansas Game and Fish Commission (scientific collection permit number 041220191). We thank A. Meinders, M. C. Swartwout, and I. N. Bertels for helping with data collection. We thank J. Woolbright and the Arkansas Natural Heritage Commission for site access and logistical support. We also thank J. Ortega, L. Kamees, and C. L. Brewster for study design and methodological advice. This project was supported by the National Science Foundation Research Experience for Undergraduates program (NSF REU award DBI2061120), a State Wildlife Grant from the Arkansas Game and Fish Commission (AGFC; RG-2708), and the University of Arkansas.

LITERATURE CITED

- [AGFC] ARKANSAS GAME AND FISH COMMISSION. 2015. Arkansas Wildlife Action Plan. Arkansas, USA.
- BATES, D., M. MÄCHLER, B. BOLKER, AND S. WALKER. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- BEAUPRE, S. J., AND F. ZAIDAN. 2012. Digestive performance in the timber rattlesnake (*Crotalus horridus*) with reference to temperature dependence and bioenergetic cost of growth. *Journal of Herpetology* 46: 637–642.
- BREWSTER, C. L., S. J. BEAUPRE, AND J. D. WILLSON. 2018. Habitat loss and local extinction: linking population declines of Eastern collared lizards (*Crotaphytus collaris*) to habitat degradation in Ozark Glades. *Journal of Herpetology* 52:352–360.
- BRYE, K. R., C. P. WEST, AND E. E. GBUR. 2004. Soil quality differences under native tallgrass prairie across a climosequence in Arkansas. *American Midland Naturalist* 152:214–231.
- BURNHAM, K. P., AND D. R. ANDERSON. 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research* 33:261–304.
- CONVERSE, S. J., AND J. A. SAVIDGE. 2003. Ambient temperature, activity, and microhabitat use by ornate box turtles (*Terrapene ornata ornata*). *Journal of Herpetology* 37:665–671.
- CURETON, J. C., A. B. BUCHMAN, R. DEATON, AND W. I. LUTTERSCHMIDT. 2011. Molecular analysis of hybridization between the box turtles *Terrapene carolina* and *T. ornata*. *Copeia* 2011:270–277.
- DE NARDO, D. F., T. E. ZUBAL, AND T. C. HOFFMAN. 2004. Cloacal evaporative cooling: a previously undescribed means of increasing evaporative water loss at higher temperatures in a desert ectotherm, the Gila monster *Heloderma suspectum*. *Journal of Experimental Biology* 207:945–953.
- DO AMARAL, J. P. S., G. MARVIN, AND V. H. HUTCHISON. 2002. Thermoregulation in the box turtles *Terrapene carolina* and *Terrapene ornata*. *Canadian Journal of Zoology* 80:934–943.
- DURRE, T., K. R. BRYE, L. S. WOOD, AND E. F. GBUR. 2019. Soil moisture regime and mound position effects on soil profile properties in a native tallgrass prairie in northwest Arkansas, USA. *Geoderma* 352: 49–60.
- FOX, J., AND S. WEISBERG. 2019. An R Companion to Applied Regression. 3rd ed. Sage, USA.
- GRANT, B. W., AND A. E. DUNHAM. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology* 69:167–176.
- HARRISON, X. A., L. DONALDSON, M. E. CORREA-CANO, J. EVANS, D. N. FISHER, C. E. GOODWIN, B. S. ROBINSON, D. J. HODGSON, AND R. INGER. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* 6:e4794.
- LAKENS, D., AND A. R. CALDWELL. 2021. Simulation-based power analysis for factorial analysis of variance designs. *Advances in Methods and Practices in Psychological Science*. doi:10.1177/2515245920951503.
- LEGLER, J. M. 1960. Natural history of the ornate box turtle, *Terrapene ornata ornata* Agassiz. University of Kansas Publications, Museum of Natural History 11:527–669.
- R CORE TEAM. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- REDMER, M., AND M. J. PARRIS. 2005. Family Ranidae. Pp. 526–599 in M. J. Lannoo, (ed.), *Amphibian Declines: The Conservation Status of United States Species*. University of California Press, USA.
- RADZIO, T. A., AND M. P. O'CONNOR. 2017. Behavior and temperature modulate a thermoregulation–predation risk trade-off in juvenile gopher tortoises. *Ethology* 123:957–965.
- REAGAN, D. P. 1974. Habitat selection in the three-toed box turtle, *Terrapene carolina triunguis*. *Copeia* 1974:512–527.
- REPSNIDER, J. M., J. STRICKLAND, AND F. J. JANZEN. 2012. Home range and site fidelity of imperiled ornate box turtles (*Terrapene ornata*) in northwestern Illinois. *Chelonian Conservation and Biology* 11:78–83.
- ROBERTSON, E. P., E. P. TANNER, R. D. ELMORE, S. D. FUHLENDORF, J. D. MAYS, J. KNUTSON, AND S. R. LOSS. 2021. Fire management alters the thermal landscape and provides multi-scale thermal options for a terrestrial turtle facing a changing climate. *Global Change Biology* 28:782–796.
- RUTHERFORD, A. 2011. ANOVA and ANCOVA: A GLM approach. Wiley, USA.
- SADEGHAYOBI, E., S. BLAKE, M. WIKELSKI, J. GIBBS, R. MACKIE, AND F. CABRERA. 2011. Digesta retention time in the Galápagos tortoise (*Chelonoidis nigra*). *Comparative Biochemistry & Physiology Part A: Molecular and Integrative Physiology* 160:493–497.
- SAMPSON, F., AND F. KNOPE. 1994. Prairie conservation in North America. *Bioscience* 41:418–421.
- STONE, M. D., AND D. MOLL. 2009. Abundance and diversity of seeds in digestive tracts of *Terrapene carolina* and *T. ornata* in southwestern Missouri. *Southwestern Naturalist* 2009:346–350.
- STURBAUM, B. A. 1981. Responses of the three-toed box turtle, *Terrapene carolina triunguis*, to heat stress. *Comparative Biochemistry & Physiology Part A: Physiology*, 70:199–204.
- STURBAUM, B. A., AND M. L. RIEDESEL. 1974. Temperature regulation responses of ornate box turtles, *Terrapene ornata*, to heat. *Comparative Biochemistry & Physiology Part A: Physiology* 48:527–538.
- WEBB, J. K., R. SHINE, AND R. M. PRINGLE. 2005. Canopy removal restores habitat quality for an endangered snake in a fire suppressed landscape. *Copeia* 2005:894–900.

Accepted: 8 October 2022.

Published online: 28 March 2023.