Effect of Sex and Personality on Thermoregulatory Behaviors and Microhabitat Selection of Brumation Sites in Fall and Winter Months in Eastern Box Turtles (*Terrapene carolina carolina*)

By
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ABSTRACT

Consistent individual variation in behavior within a population is commonly referred to as personality across animal taxa. As our understanding of animal personality increases, researchers can gain better insight into the way personality may affect different behaviors. Research has shown correlations between personality and productivity, population density, stability of populations, dispersal, colonization, distributions within habitats, and disease transmission. Because personality apparently impacts so many attributes of the lives of various species, research on personality variation in different species is especially important. Eastern Box Turtles, *Terrapene carolina carolina*, are a long-lived species with a distribution across much of the eastern United States. Past studies have shown that Eastern Box Turtles exhibit two personality variations, bold and less bold, which affect movement rate, home range size, date of emergence from brumation, and thermoregulation. Research has also shown that sex may affect home range size, movement distances, utilization of developed habitats, and thermoregulation. Little research, however, has been done to determine the effect of personality and sex on either thermoregulation during fall and winter, or brumation site selection in Eastern Box Turtles. This study examined whether sex and boldness influence thermoregulation during the fall and winter, and brumation site microhabitat selection in wild Eastern Box Turtles. Bold turtles maintained higher body temperatures than less bold turtles at all times of day regardless of sex. Females maintained higher body temperatures when compared to males when boldness was not accounted for. When the interaction of sex and boldness was measured, the four combinations of sex and boldness (e.g., bold female, bold male, less bold female, less bold male) in turtles were found to be significantly different from one another in how they thermoregulated. Bold male turtles exhibited the warmest temperatures overall, whereas less bold male turtles exhibited the coolest temperatures overall. Bold and less bold male box turtles differed dramatically in how they thermoregulated (average maximum difference of ~1.5°C); bold and less bold females also differed but the difference was more subtle (average maximum difference of ~0.5°C). Brumation site microhabitat selection was not found to differ for sex, boldness, or their interaction. This likely means that brumation site selection is dependent on factors other than sex or personality or could be relatively stochastic within their tolerable range. Further research into the interactions of sex and boldness on thermoregulatory behaviors in different portions of the range of Eastern Box Turtles would help elucidate the proximate mechanisms and functional consequences of personality.

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INTRODUCTION

The consistent individual variation in behavior within a population of animals is commonly referred to as personality (Carter et al., 2010; Réale et al., 2007, 2010; Wolf and Weissing, 2012). To standardize terminology used to describe animal temperament, Réale et al. (2007) produced a list of terms with their ecological situation for use, tests for each characteristic, operational definitions, examples of studies demonstrating the terms, and the ecological validity of each term. The boldness trait is defined as having phenotypes including bold, docile, shy, tame, untame, fearful, and unfearful, and there are various tests that can be used to determine the boldness of a given individual (Réale et al., 2007).

Wolf and Weissing (2012) review the ecological implications of different personalities across species of animals, and they provide a list of reasons for why behavioral differentiation might evolve within a species. Focusing specifically on personality differentiation within Bluegill sunfish (*Lepomis macrochirus*), Wolf and Weissing (2012) suggest that having a variety of personalities within a species can affect productivity and population density, stability of populations, dispersal, colonization, distributions within habitats, disease transmission, and other demographic parameters. Productivity and population density refers to a population having behavioral differentiation to increase carrying capacity and productivity within that population to avoid competition and provide for better division of labor through behavioral complementation (Wolf and Weissing, 2012). The stability of a population, dispersal, colonization, and distribution within a habitat are driven by behavioral differentiation aiding in persistence of that population in habitats by allowing for different personalities to function in different niches to support the overall population (Wolf and Weissing, 2012). Behavioral differentiation also assists in preventing disease transmission by keeping different phenotypes and genotypes within the population, which makes it harder for disease to affect the entire population (Wolf and Weissing, 2012).

Previous research on lower vertebrates has shown that boldness may manifest itself in several behavioral traits and may have a variety of costs and benefits. For example, Carter et al. (2010) found Agamas in Namibia (Namibian Rock Agamas, *Agama planiceps*) do not habituate to repeated behavioral assays, thus the personality type does not change based on number and frequency of behavioral assays, suggesting personality is a fixed trait over the lifespan of an individual. They also found that bolder Agamas spend more time performing conspicuous behaviors than those that were less bold (Carter et al., 2010). Bolder Agamas also have larger home ranges and a higher risk of predation (Carter et al., 2010). Bold male Zebrafish (*Danio rerio*) had higher reproductive success than middle and shy male Zebrafish (Ariyoma and Watt, 2011). Given the prevalence of middle (i.e., intermediate personality) and shy personalities within the population, there must be certain costs associated with the bold personality that allows these other personalities to persist, however, those costs are currently unknown (Ariyoma and Watt, 2011). Because different personality types in many species result in differences in a variety of behaviors, it could be assumed that personality differences in Eastern Box Turtles (*Terrapene carolina carolina*) may also affect behaviors and, consequently, population dynamics. At present, the costs and benefits of boldness in box turtles are largely unknown.

Because of declining population sizes throughout their distributional range, Eastern Box Turtles are a species of concern in Tennessee. Conservation efforts would be improved by a more complete understanding of their thermoregulatory behaviors and overwintering site selection, as these behaviors are vital for many physiological functions and successful survival over winter. Furthermore, despite several studies of personality in box turtles by different research groups, little is known about the costs and benefits of personality in this species. Therefore, I studied the effect of personality and sex on thermoregulation and microhabitat selection in the fall and during brumation of Eastern Box Turtles (*Terrapene carolina carolina*). Specific objectives of the study include (1) determine the sex and boldness of all individuals on the study, (2) determine if bold and less bold turtles have different thermoregulatory behaviors, (3) determine if male and female turtles have different thermoregulatory behaviors, (4) determine if sex and boldness interact in behaviors, and (5) test whether personality or sex affect brumation site selection. To do so, I located the turtles once weekly to determine microhabitat use throughout the fall months and the brumation site selected, ran five behavioral tests on each turtle to estimate its personality (bold vs less bold), described the microhabitat selected for brumation by each turtle, and recorded the shell temperatures of each turtle as well as the availability of environmental temperatures.

METHODS

Study site and subjects

In this study, male and female adult Eastern Box Turtles (*T. carolina carolina*) were sampled from a 30.5 ha forested wetland preserve in Murfreesboro, Tennessee, USA called Nickajack Trace and Blackfox Wetlands (Figure 1). Seven turtles (4 male, 3 female) were captured, tracked, and tested in behavioral assays in the first field season between mid-September of 2022 and February of 2023. Twelve turtles (5 males and 7 females) were captured, tracked, and tested in behavioral assays in the second field season between September of 2023 and January of 2024. Subjects in each field season were tested 5 times over an average of 25 days (range: 19-35 d) to evaluate consistencies in their behavior (i.e., personality) during and immediately following handling. A minimum 5-day recovery period occurred between behavioral assays following the initial two tests to reduce the likelihood that turtles would habituate to interactions with the investigator (similar to Kashon and Carlson, 2018 and Warren, 2022). All capture and behavioral assays occurred between 9:00 and 16:00 CDT. To prevent the spread of disease, fresh nitrile gloves were used when handling each turtle, and all equipment was cleaned between use by applying a 5% bleach solution for at least 60 sec.

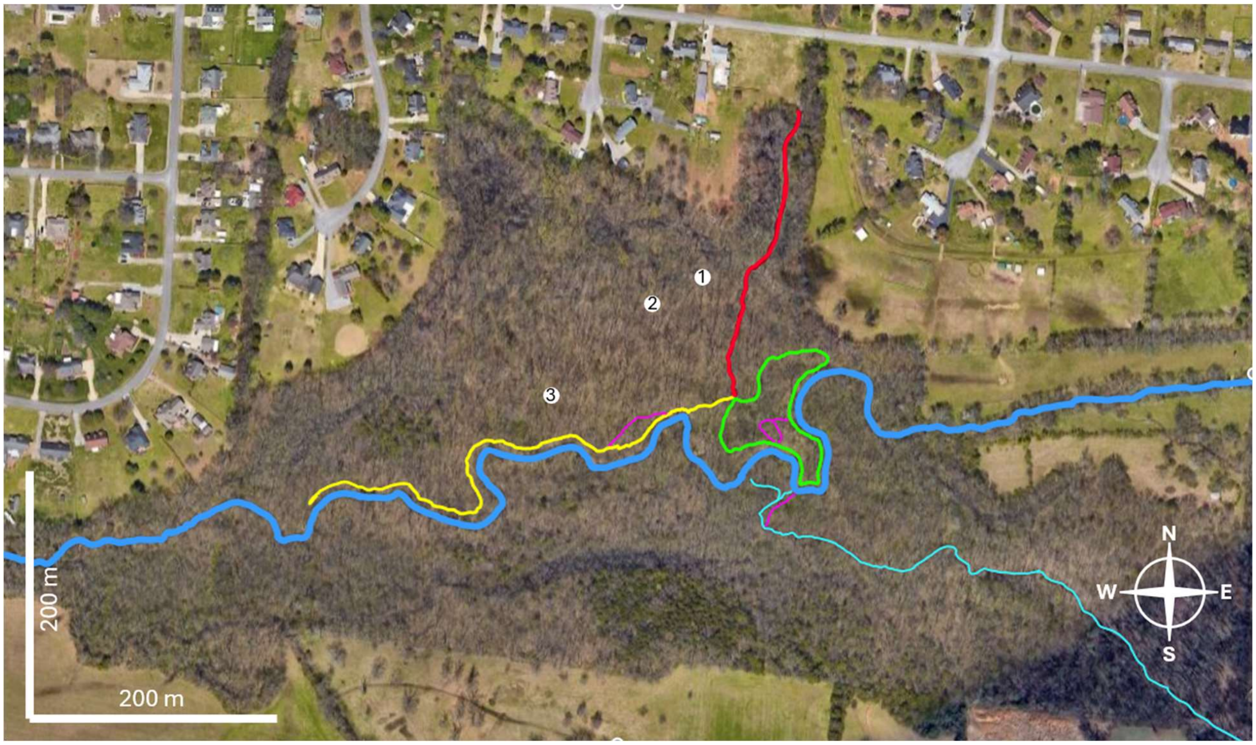

Figure 1 An approximate Nickajack Trace and Blackfox Wetlands map. The main blue line is Lytle Creek. The red, yellow, and green lines are all main trails. The purple lines are minor trails. The teal line is a creek bed that feeds into Lytle Creek when precipitation is high. Point 1 is field station 1 from the first field season. Point 2 is field station 1 from the second field season. Point 3 is field station 2 from both field seasons.

Initial capture and assay conditions

The selection of turtles was random (haphazard) and was based on a visual search at the field site. After capture, each turtle was sexed by examining eye color, tail length, and degree of concavity of the plastron (Kiester and Willey, 2015). Each turtle was identified by three notches carved along the marginal scutes of the carapace, where each scute represents a corresponding letter in the alphabet. If a turtle had previously been used in a study, the notches were already present, and I reused the identification code for that turtle. If the turtle had no notches, three notches were carved in the scutes using a triangular file to give the turtle a unique identification code. During the 2022-2023 field season, I studied seven turtles, six of which were previously marked.

During the 2023-2024 field season, I studied 12 turtles, eight of which were previously marked. No turtle was used in both field seasons, so my data is for 19 individuals.

I processed each turtle at the initial capture. Processing included measuring the straight-line length of the carapace, straight-line width of the carapace, plastron length anterior to the hinge, plastron length posterior to the hinge, and plastron width at the hinge. All measurements were taken to the nearest 0.5 mm. I also weighed each turtle to the nearest gram and counted the toes on the front and hind limbs. I estimated the age of each turtle by counting the annuli of at least two scutes on the carapace and averaging the numbers to get an estimate of age in years (Budischak et. al., 2006; Ewing, 1939). I also noted environmental conditions at the capture site of each turtle, including capture time, air temperature at 0.5 m high, substrate temperature at 5 cm below ground level, sky conditions (see below), GPS coordinates, habitat type (forest, field, trail, edge), and microhabitat type (on leaves, grass, rock, soil, or water). The first of five behavioral assays was run after processing a turtle.

Following the initial behavioral assay (see below), each turtle was placed in its own plastic bucket, brought into an indoor, temperature-controlled environment, and kept overnight (< 18 hours) within their buckets. During the time in captivity, the turtles were handled to take photographs and to attach radio transmitters (RI-2B Holohil 19 Systems, Ontario Canada) and a Thermochron® iButton temperature data logger (DS1921G, Maxim Integrated, San Jose, California) to their shells using a two-part epoxy. Radio transmitters and iButtons® were attached onto the posterior end of the carapace, oriented largely on each of the posterior-most costal scutes, just above the posterior marginal scutes (Figure 2). The portion of the iButtons not in contact with the carapace were covered with a black rubber coating (Plasti Dip®, Blaine, Minnesota) while the underside of both the transmitters and iButtons were attached directly to the carapace using a thin layer of Epoxy (Loctite®, Westlake, Ohio). The following morning, I returned turtles to their capture sites (± 1 m) where they underwent their second behavioral test and subsequent release.

Figure 2 Posterior turtle carapace with radio transmitter attached on the left and an iButton attached on the right.

After release, I began regular tracking and behavioral tests for three additional captures with at least a 5-day recovery period between each test. In general, all five behavioral tests were completed on average in 25 days from initial capture (range: 19–35 d). Personality tests were ideally collected every seven days but varied between 5 and 28 days due to inclement weather, availability to track, and turtle accessibility in the field. Two turtles exhibited signs of illness (e.g., swollen eyes, lethargy, discharge from eyes and nose) in separate field seasons, which resulted in a 4-week recovery period between tests for those turtles until they appeared healthy. I replaced one turtle in the study that had died for unknown reasons (i.e., had no visible signs of injury). I began testing the replacement turtle one month later than the other turtles; I tested the personality of the replacement turtle only four times before she began brumation. Other test conditions were recorded at each capture event including an infrared temperature reading (Etekcity Infrared Thermometer 749, Anaheim, California) on the uppermost portion of the shell covered in shade, the time of day, and a ranking of sky conditions between 0 and 3 using the following criteria: 0 = clear skies, 1 = partly cloudy or variable skies, 2 = cloudy overcast with few if any breaks between clouds, and 3 = rainy.

Behavioral assays

I conducted the first behavioral assay of each turtle at the time of initial capture. I handled each turtle for three minutes while processing the turtle (e.g., size, weight, approximate age, etc.). I recorded any defensive behaviors observed during processing, including number of snaps, bites, urination events, and defecation events. I also noted and recorded the amount of time spent air walking (e.g., head and limbs fully out of the shell and limbs moving as though walking) during the 3-min handling period. After release, I recorded the following behaviors during a 10-minute observation period while standing motionless from a distance of approximately 5-8 m from the turtle: (1) time until the eyes extended beyond the perimeter of the shell, (2) time for the entire head to extend beyond the perimeter of the shell, (3) time for the wrists to extend beyond the perimeter of the shell, (4) time for the turtle to move two body lengths, and (5) time for the turtle to move one meter.

Boldness classification

Boldness classification was determined using a hierarchical cluster analysis in R, using a Euclidean distance matrix generated from data that was first centered and scaled to unit variance (via the functions “scale”, “dist” and “hclust”). This analysis used data from the 3 min handling period including the average time spent in a closed shell and average score for air walking events (0 = no air walking events, 1 = air walking occurred). The analysis also used data from the 10 min observation period including average latency for eyes to extend beyond the shell perimeter, average time it took to move two body lengths, and average score of whether the turtle moved one meter (0 = did not move 1 m, 1 = did move a meter). These criteria were selected based on the compiled behavior method of boldness classification used in other studies (Cassola et al., 2020; Warren, 2022). The cluster analysis was selected as it reduces bias in the classification process (Ketchen and Shook, 1996).

Removal of radio transmitters and temperature loggers

I removed and replaced iButtons every 42-84 days, depending on the storage capacity. I used a pocketknife to remove iButtons and I peeled any additional epoxy off from the shell. After the turtle had not moved from a brumation site for 21 days and if the turtle's transmitter and iButton were visible (i.e., not buried), then I removed both the transmitter and iButton using the method previously described. If the turtle was buried, then I waited until it voluntarily emerged in the spring (typically April) to remove the transmitter and iButton. Throughout the duration of the study, all transmitters and iButtons remained firmly attached to the carapace, and no obvious alterations to the underlying shell were caused by the adhesive.

Temperature data collection and differential estimates

To estimate the range of available environmental temperatures, I set up two environmental temperature monitoring stations at my field site. Both field stations were at least 30 meters off the main trail and were approximately 100 m apart from one another but in a similar forested section of the field site (Figure 1). I placed four iButtons at each station that recorded the ambient temperature every thirty minutes for the duration of the study. I placed one iButton 1 m above the ground, a second iButton placed 5 cm above the ground, a third at ground level, and a fourth buried 10 cm underground. As the iButtons reached data storage capacity, I replaced them with new iButtons. After all leaves had fallen at the field site in the first field season, I placed foil over all above ground iButtons to prevent direct sunlight from impacting the temperatures being collected. In the second field season, foil was placed over all above ground iButtons from the start of the season to reduce inaccurate reading from direct sunlight that occurred in some of the data from the first field season. Inaccurate readings were determined by examining other environmental temperatures, both from the field stations and turtles and from the National Weather Service and omitting observations that were significantly too high or too low for the given day and time.

Brumation site microhabitat measures

When a turtle had been found in the same location for three consecutive weeks, brumation site microhabitat data was collected. I placed a 1 meter by 1 meter square constructed of PVC pipe over the turtle, with the center of the square positioned as close to the approximate center of the carapace as possible. I used a digital camera oriented parallel to the ground and held approximately 1.8 m above the ground to photograph the entire square meter of space around the turtle. I also used a digital camera to photograph the sky and tree cover directly above the turtle. I recorded air temperature, soil temperature next to the turtle, soil moisture content adjacent to the turtle (XLUX T10 Bodentester Soil Moisture Meter, Bantian, China), temperature of the carapace, and the sky conditions. Furthermore, I measured turtle depth from ground level to the highest point of the carapace and depth of leaf cover directly over the turtle. Lastly, I measured the horizontal distance between the center of the turtle's carapace to any branches, trees, or woody plants with a diameter of > 2 cm within the square.

Statistical analyses

I analyzed and plotted data using the statistics programming language R within the IDE RStudio (R Core Team, 2024). For all statistical analyses, $\alpha = 0.05$. Statistical significance was determined by evaluation of p-values for generalized additive mixed models, ANOVA, and MANOVA.

Turtle demographics data was analyzed using an ANOVA in R. We tested whether mean carapace length or body mass differed by sex or boldness, using a two-way ANOVA with interaction for each variable.

I analyzed turtle temperature data using a generalized additive mixed model (GAMM), which allows the dependent variable to vary as a non-linear function of the independent variables (e.g., with cyclical peaks and lows in the day and night), and account for random effects (e.g., repeated measurements per turtle). The model included body temperature as the response variable, boldness and sex as the main predictor variables, and accounted for time of day (in hours), date, turtle ID, and year as random variables. We used a tenser (function "ti") for the fixed effect of the time of day variable, and allowed temperature "smooths" (i.e., non-linear trends) to vary with sex and boldness terms as the temperature data is non-linear. We used a thin-plate spline smoothing term for day of year to account for seasonally varying temperatures. Year and turtle ID were treated as random effects (function "s", basis = "re") to account for variation over sampling periods or based on other factors varying between the individual turtles. All gamm analyses were implemented using the R package "mgcv" using the R function "gamm" and its associated summary functions (Bates et al., 2015; van Rij et al., 2022; Wood et al., 2016). To utilize dates in gamm analyses, the package "lubridate" was used to convert the dates into a numeric format (Grolemund and Wickham, 2016).

Brumation start data was analyzed using an ANOVA in R. The package "lubridate" was used to convert the dates into a format that R would recognize (Grolemund and Wickham, 2016). Habitat data was analyzed using custom R code that utilized the digital images from the brumation site of each turtle and quantified the proportion of dead foliage, live foliage, woody material, and sky within the square meter. Specifically, we used the package "recolorize" (Weller, 2021) to recategorize pixel color values into a set of discrete categories, which we mapped to specific attributes previously stated (e.g., life foliage). We found that branches in particular were hard to discriminate using this approach and manually recolored these to a highly contrasting color and increased the color saturation prior to analysis (Figure 3). We used a hierarchical cluster analysis of a 5X5 binned color space of a combined set of sample ground photos to select color groupings (function "recolorize" and "recluster"), and applied classifications to all ground images using the function "imposeColors". For sky images, we found that a k-means based classification was effective for discriminating sky from canopy cover (k = 3, 2 color categories for trunk/branches). A MANOVA was then utilized to test for differences in those proportions between sexes and personalities.

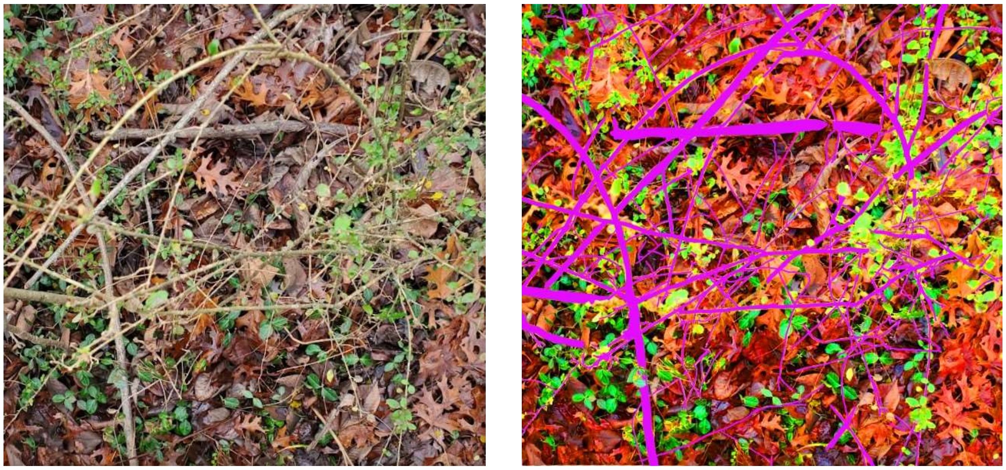

Figure 3 Brumation site ground photo sample before editing colors (left) and after editing colors (right) to differentiate dead foliage, live foliage, and wood colors more clearly in the analysis.

RESULTS

Turtle Demographics

In the first field season, 7 turtles were tracked (3 females, 4 males). In the second field season, 12 turtles were tracked (7 females, 5 males). Across both field seasons, the smallest straight line carapace length was 113.0 mm and the largest was 145.5 mm (Table 1). Turtle body mass at the start of the study ranged from 265-500 grams. Sex, boldness, and their interaction had no effect on carapace length or body mass. Turtles ranged from an estimated 10-19 years of age.

Table 1 Turtle demographics across both field seasons. CL represents carapace length, measured in mm. Body mass is measured in grams. Age is estimated from best annuli count. Date captured is the date the turtle joined the study, and date finished is when the turtle was immobile for three full weeks at its brumation site, at which point it was removed from the study. *Subject died during the study

Turtle	**CL**	**Body Mass**	**Est. Age**	**Captured**	**Finished**	**Boldness**	**Sex**	**Year**
APQ	130.0	500	17	13 September 2022	18 December 2022	B	F	1
MNW	126.0	285	14	23 September 2022	18 December 2022	B	F	1
KNX	127.5	440	12	15 September 2023	01 December 2023	B	F	2
IJV	142.5	470	14	15 September 2023	06 December 2023	B	F	2
HKW	127.0	360	13	14 October 2023	15 December 2023	B	F	2
IKW/X	136.0	400	12	08 September 2023	08 January 2024	B	F	2
IJW*	128.0	354	19	08 September 2023	-----	B	F	2
ACM	133.0	400	15	23 September 2022	18 December 2022	B	M	1
DGR	113.0	320	12	13 September 2023	08 January 2024	B	M	2
IJO	120.0	265	13	08 September 2023	01 December 2023	B	M	2
BCP	145.5	430	17	15 September 2023	25 November 2023	B	M	2
BNQ	138.5	435	14	14 September 2023	24 November 2023	B	M	2
KMP	131.5	455	13	23 September 2022	18 December 2022	N	F	1
BNW	128.5	350	10	14 September 2023	15 December 2023	N	F	2
HNQ	116.0	362	16	12 September 2023	15 December 2023	N	F	2
IMN	124.0	327	12	27 September 2022	18 December 2022	N	M	1
AJN	125.0	350	13	20 September 2022	11 December 2022	N	M	1
JNW	126.0	360	11	20 September 2022	08 November 2022	N	M	1
CKU	121.5	360	14	13 September 2023	25 November 2023	N	M	2
Range:	**113.0-145.5**	**265-500**	**10-19**					

Personality Categorization

Twelve turtles were categorized as ‘bold’, and seven turtles were classified as ‘less bold’ by the k-means cluster analysis across both field seasons (Figure 4). Only one turtle, HNQ, varied slightly in classification in the iterations of the analysis. HNQ, however, fell into the ‘less bold’ category more often than in the ‘bold’ category.

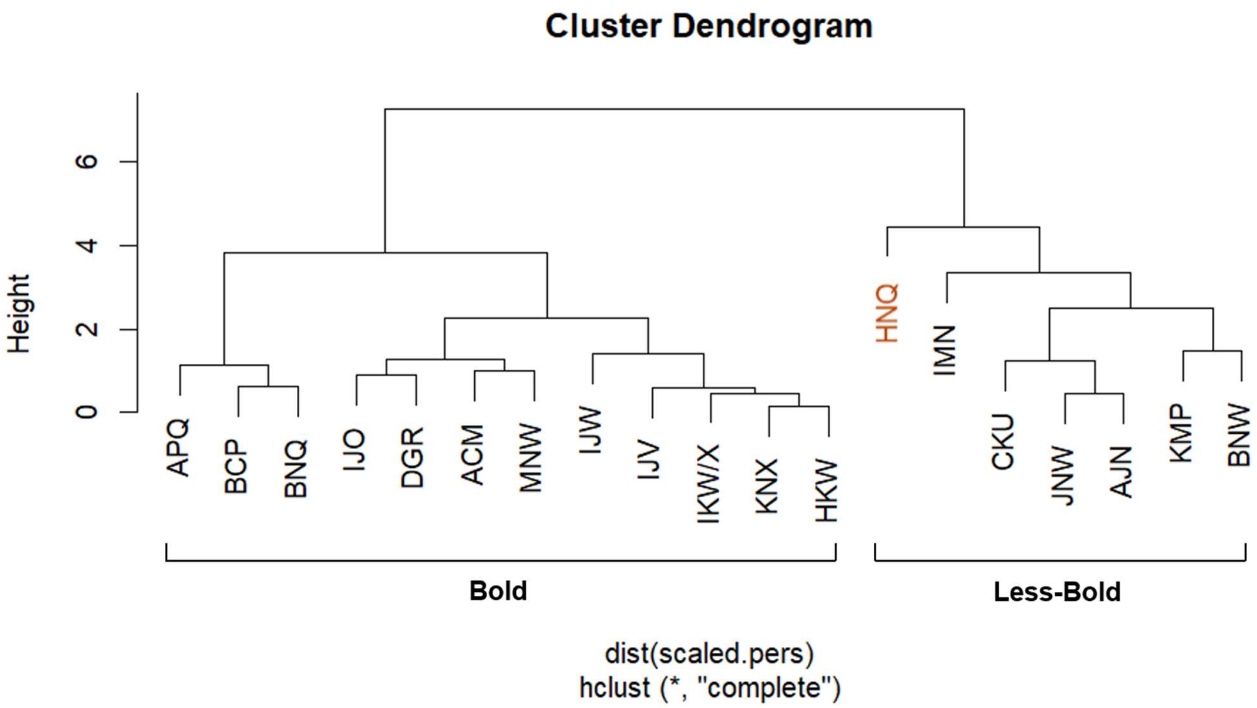

Figure 4 Cluster dendrogram for boldness classification for the 19 turtles from the study. The left half of the dendrogram represents the bold group of turtles and the right half of the dendrogram represents the less bold group of turtles. The branch labels correspond to the identification codes for each turtle. The height axis displays the distance between individuals and clusters. HNQ varied in classification but was classified as less bold more frequently.

Body Temperature

The highest environmental temperature recorded in Year 1 was 30.5°C on 21 September 2022, and in Year 2 it was 29.0°C on 26 September 2023. The lowest environmental temperature for Year 1 was -18.5°C on 23 December 2022, and for Year 2 it was -7.5°C on 29 November 2023.

As expected, temperatures peaked in mid-afternoon (12:00-16:00 CDT) and were coolest in the early hours of the morning (6:00-9:00 CDT).

Temperatures from the turtles show that the warmest temperature a turtle reached in Year 1 was 37.0°C on 17 October 2022 and in Year 2 it was 38.0°C on 01 October 2023. The coldest temperature a turtle reached during Year 1 was -0.5°C on 21 November 2022, and in Year 2 it was 0.0°C on 02 November 2023. Body temperatures of turtles essentially mirrored environmental temperatures, with a warmest temperature recorded in mid-afternoon and a coolest temperature recorded in the early morning hours.

All turtle temperature data, including that of IJW who died during the study, were used for the temperature analyses (Figures 5-8). With sex excluded from analyses (but still accounting for date, ID, etc), bold and less bold turtles have significantly different temperature "smooths", with bold turtles maintaining consistently higher body temperatures than less bold turtles (f range = 513.7-1106.7, df range = 11.47-12.54, $p < 0.01$) (Figure 5). The maximum difference in body temperatures (about 2.0° C) between bold and less bold turtles tended to occur in the mid-afternoon (around 14:30 CDT).

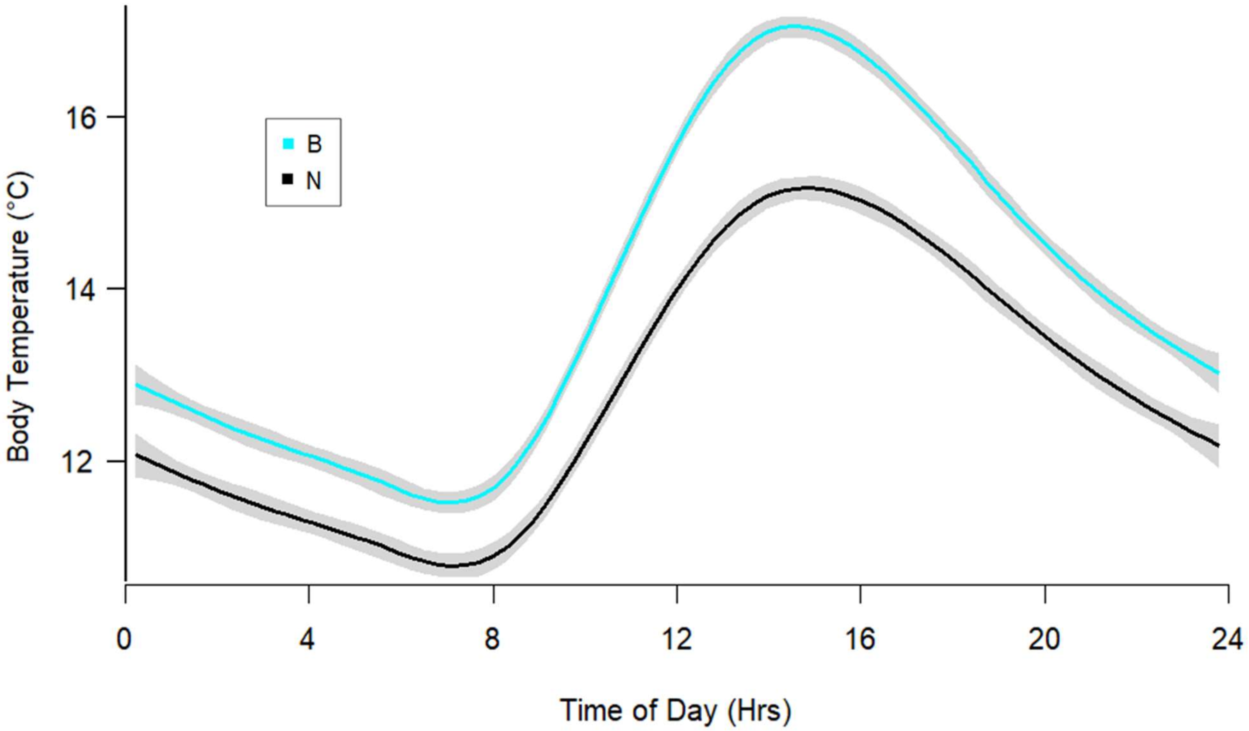

Figure 5 Smooths for bold and less bold turtle body temperatures where B is bold (n = 12) and N is less bold (n = 7). The boldness curves are significantly different from one another ($p < 0.01$). Sex was excluded from this analysis.

Without boldness considered, male and female turtles have significantly different temperature smooths, with female turtles maintaining higher body temperatures than male turtles (F range = 656.6-962.9, df range = 11.89-12.36, $p < 0.01$; Figure 6). The maximum difference in body temperatures (about 1.2°C) between male and female turtles also tended to occur in the mid-afternoon (around 14:30 CDT).

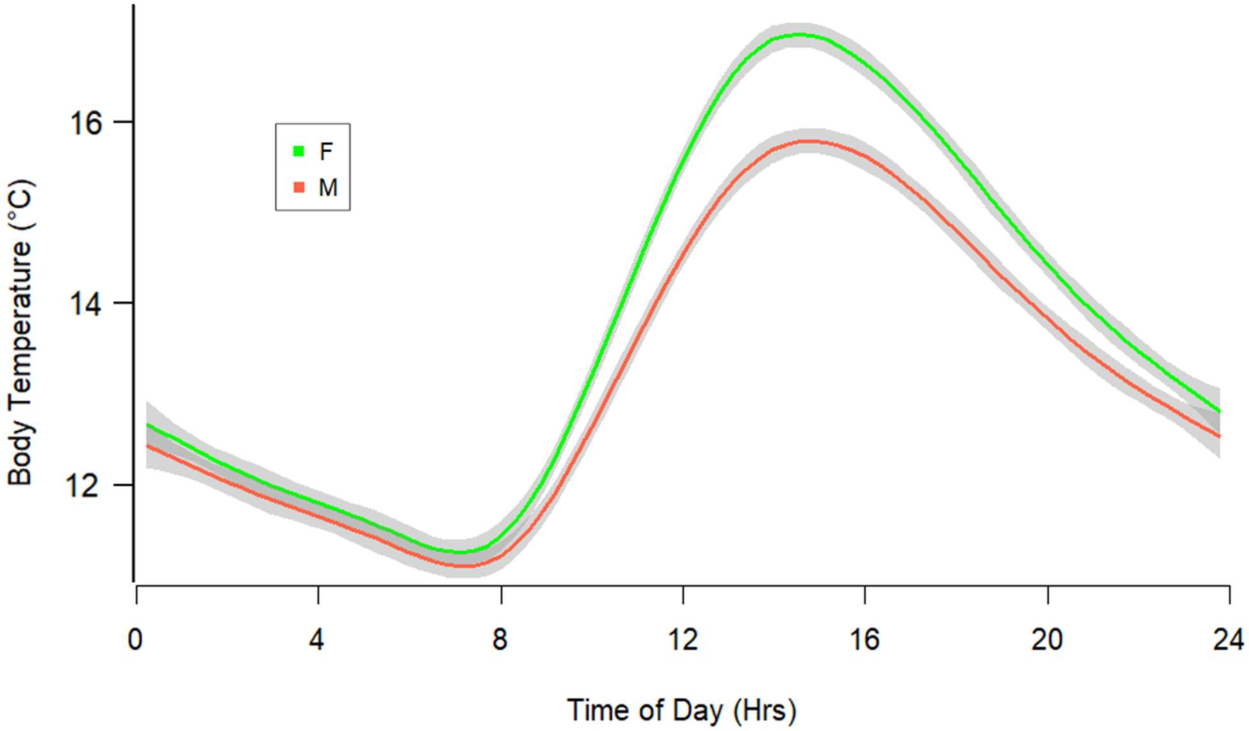

Figure 6 Smooths for female and male turtle body temperatures where F is female (n = 10) and M is male (n = 9). The boldness curves are significantly different from one another ($p < 0.01$). Boldness was excluded from this analysis.

The smooths of the combinations of boldness and sex are all at the same intercept in Figure 6, allowing for the slope of the smooths to be compared. Less bold males (shown in blue) have a flatter curve, meaning there is less variation in temperature throughout the day compared to the other groups (Figure 7). Although the other three groups are more similar in shape, they all significantly differ from one another ($p < 0.01$). Bold turtles, regardless of sex, tend to have higher peaks during the day and lower peaks at night than less bold turtles. Less bold turtles tend to have flatter smooths and less variation in temperatures throughout the day than bold turtles.

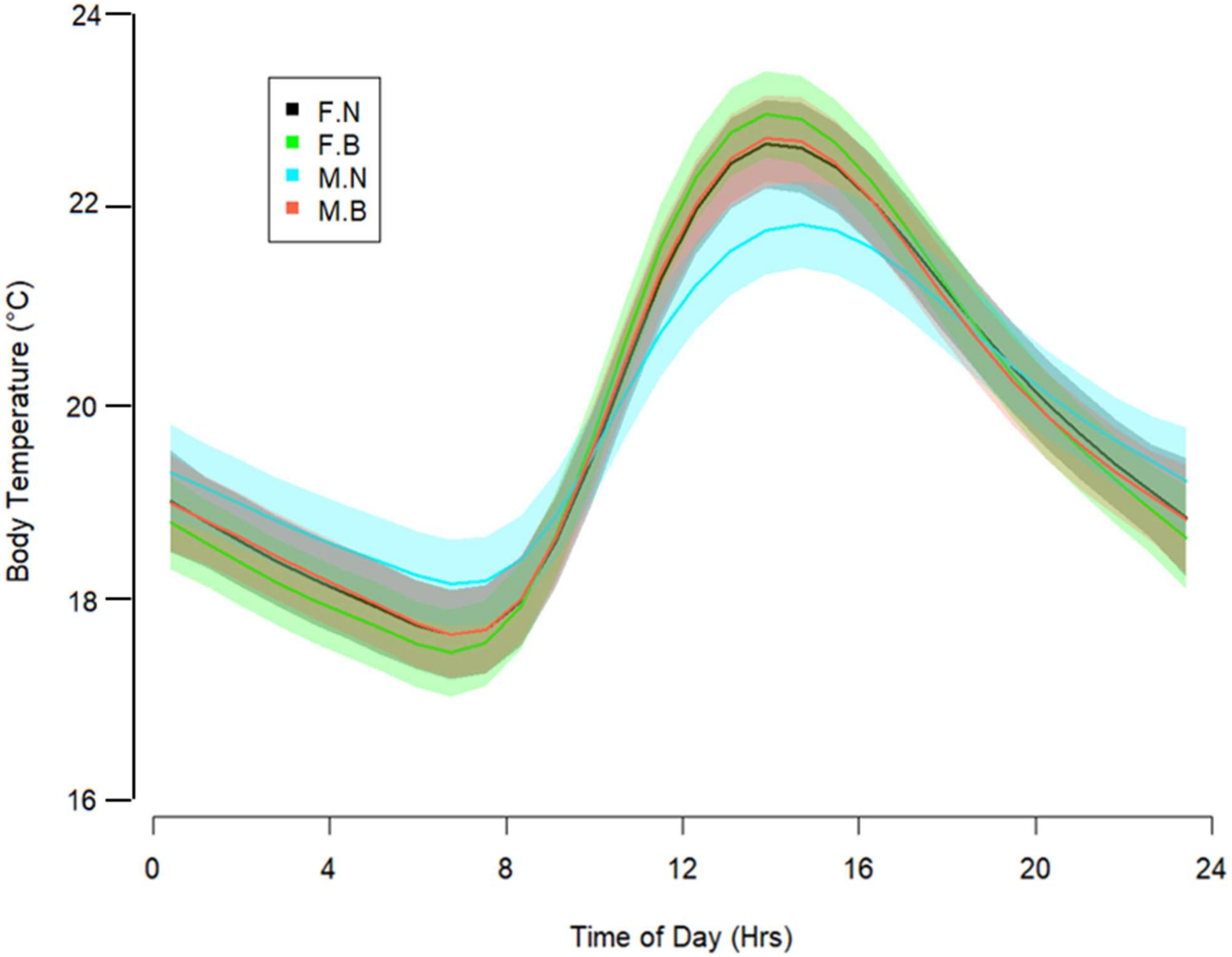

***Figure** 7* Smooths for the combinations of boldness and sex on body temperature where F.N is less bold females (n = 3), F.B is bold females (n = 7), M.N is less bold males (n = 4), and M.B is bold males (n = 5). Intercepts are not present to show differences in the smooths. All smooths are significantly different from one another ($p < 0.01$).

When comparing smooths between sexes and boldness categories, less bold males exhibited significantly lower body temperatures than all other sex and boldness combinations (F range = 308.3-693.5, df range = 10.72-11.92, $p < 0.01$; Figure 8). The smooth for less bold females shows temperatures were different from bold turtles (generally lower), especially in the afternoon, and greater than less bold males (F range = 248.9-687.5, df range = 10.15-11.91, $p < 0.01$). The smooth for bold males shows temperatures were greater than all other combinations of sex and boldness (F range = 243.9-672.9, df range = 10.11-11.87, $p < 0.01$). The smooth for bold females shows temperatures were different from less bold turtles (generally higher) and less than bold males (F range = 236.5-429.2, df range = 10.05-11.33, $p < 0.01$).

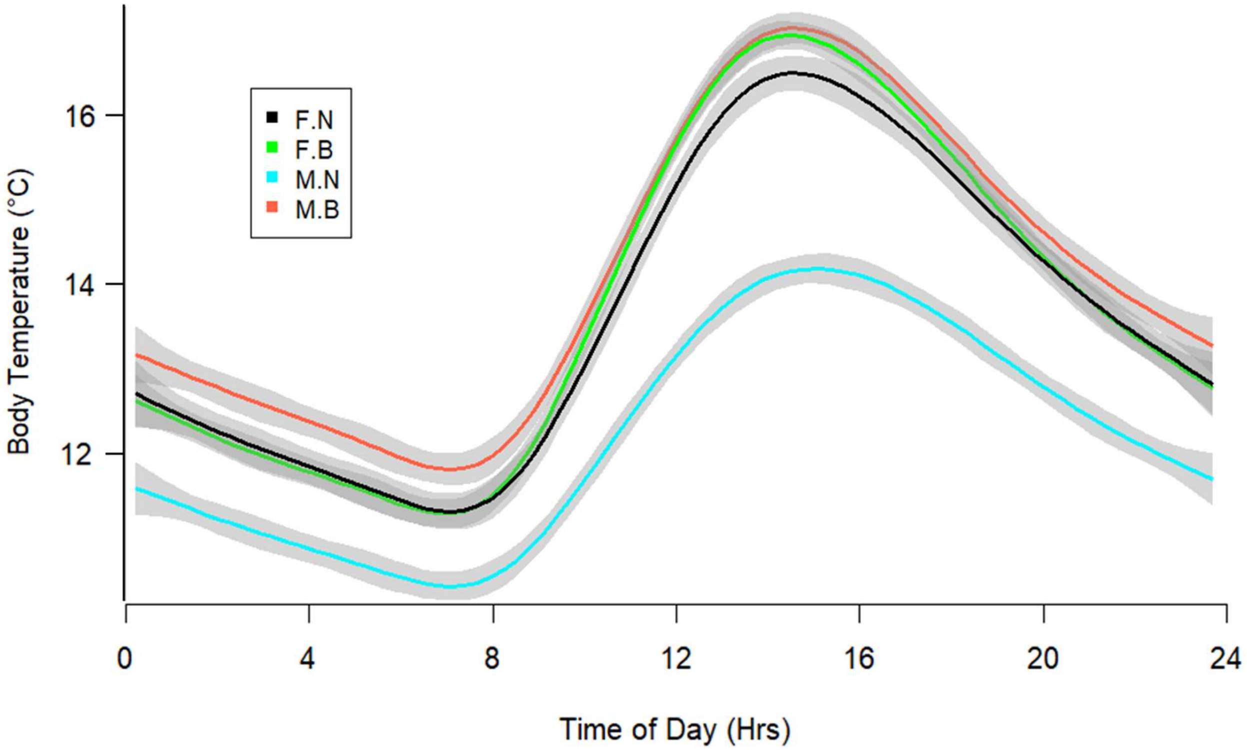

Figure 8 Smooths and intercepts for the combinations of boldness and sex on body temperature where F.N is less bold females, F.B is bold females, M.N is less bold males, and M.B is bold males. All smooths are significantly different from one another ($p < 0.01$). Sample sizes are given in Figure 7.

Generalized additive mixed models indicate both sex and boldness to be significant predictors for thermoregulation trends with males having the greatest variation in temperatures based on boldness. Similar results were found when sex and boldness were tested separately.

Brumation Microhabitat

Across both field seasons, the earliest a turtle entered brumation was mid-October and the latest was mid-December (Table 2). The ANOVA of the start dates for brumation showed no significant differences for sex, boldness, or their interaction (Table 3).

Table 2 Brumation start dates for each turtle. Brumation start is the first documented date that a turtle had entered brumation.

Turtle	**Brum. Start**	**Boldness**	**Sex**	**Year**
APQ	27 November 2022	B	F	1
MNW	27 November 2022	B	F	1
KNX	10 November 2023	B	F	2
IJV	15 November 2023	B	F	2
HKW	24 November 2023	B	F	2
IKW/X	18 December 2023	B	F	2
ACM	27 November 2022	B	M	1
DGR	18 December 2023	B	M	2
IJO	10 November 2023	B	M	2
BCP	04 November 2023	B	M	2
BNQ	03 November 2023	B	M	2
KMP	27 November 2022	N	F	1
BNW	24 November 2023	N	F	2
HNQ	24 November 2023	N	F	2
IMN	27 November 2022	N	M	1
AJN	21 November 2022	N	M	1
JNW	18 October 2022	N	M	1
CKU	04 November 2023	N	M	2

Table 3 Results of the ANOVA from the values in Table 2. There is no significant difference for sex, boldness, or the interaction of boldness and sex on brumation start dates.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Boldness	1	1.17E+10	1.17E+10	1.636	0.222
Sex	1	5.52E+09	5.52E+09	0.773	0.394
Boldness:Sex	1	8.80E+09	8.80E+09	1.233	0.286
Residuals	14	9.99E+10	7.14E+09		

Across all turtles, only three were found to completely cover their carapace with > 1 cm of soil (Table 4). The remaining fifteen turtles were found in small forms with leaf litter and ≤ 1 cm of soil covering the carapace. The maximum depth of a buried turtle was 6 cm below ground level (mean depth = 0.8 cm). The maximum depth of leaf litter over a turtle was 16 cm (mean depth = 8.8 cm).

Table 4 Brumation site turtle depths. Carapace depth measures the distance from the highest point of the carapace to ground level in cm. Leaf depth measures the amount of leaf litter present over the highest part of the carapace and any soil covering the carapace in cm.

Turtle	**Carapace Depth**	**Leaf Depth**	**Boldness**	**Sex**	**Year**
APQ	0	8	B	F	1
MNW	0	12	B	F	1
KNX	0	5	B	F	2
IJV	0	6	B	F	2
HKW	0	2.5	B	F	2
IKW/X	4	3.5	B	F	2
ACM	0	11	B	M	1
DGR	1	11	B	M	2
IJO	0	16	B	M	2
BCP	< 1	9	B	M	2
BNQ	0	12	B	M	2
KMP	0	3	N	F	1
BNW	2	15	N	F	2
HNQ	0	4.5	N	F	2
IMN	0	3	N	M	1
AJN	0	10	N	M	1
JNW	6	12	N	M	1
CKU	1	15	N	M	2
Range:	**0-6**	**3-16**			

The custom R code produced the proportions of dead plant matter, live plant matter, and woody matter from the brumation site ground digital images, and the proportion of sky to tree from the brumation site sky digital images (Table 5). The MANOVA on the proportions from the brumation site ground and sky cover digital images found no significant differences for sex or boldness (Table 6).

Table 5 Proportions of dead plant matter, live plant matter, woody material, and open sky from turtle brumation site digital images. Also listed is the ID of each turtle and the sex, boldness, and field season of each turtle.

Turtle	**Dead Leaves**	**Live Plants**	**Wood**	**Sky Proportion**	**Boldness**	**Sex**	**Year**
APQ	76.13	11.73	12.14	35.83	B	F	1
MNW	94.71	0.02	5.12	69.42	B	F	1
KNX	87.03	5.06	7.91	58.46	B	F	2
IJV	83.52	3.62	12.86	63.79	B	F	2
HKW	90.97	5.17	3.85	51.11	B	F	2
IKW/X	56.17	7.54	36.27	51.58	B	F	2
ACM	69.71	13.82	16.47	36.99	B	M	1
DGR	83.79	1.08	15.12	85.20	B	M	2
IJO	89.87	6.21	3.92	47.41	B	M	2
BCP	81.14	3.37	15.48	63.87	B	M	2
BNQ	85.71	1.45	12.84	35.37	B	M	2
KMP	86.19	1.66	12.15	51.03	N	F	1
BNW	81.11	2.68	16.21	57.29	N	F	2
HNQ	94.12	0.82	5.05	53.93	N	F	2
IMN	90.10	5.70	4.20	63.66	N	M	1
AJN	62.20	15.31	22.49	46.69	N	M	1
JNW	65.49	5.51	28.97	57.70	N	M	1
CKU	83.41	3.71	12.84	37.03	N	M	2
Range:	**56.2-94.7**	**0.02-15.3**	**3.85-36.3**	**35.4-85.2**			

Table 6 Results of the MANOVA from the values in Table 5. There is no significant difference for sex, boldness, or the interaction of boldness and sex.

	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Boldness	1	0.03746	0.10703	4	11	0.9776
Sex	1	0.08016	0.23964	4	11	0.9100
Boldness:Sex	1	0.32803	1.34246	4	11	0.3149
Residuals	14					

DISCUSSION

Eastern Box Turtles have a large distribution throughout the United States, extending from southern Maine to the Florida Keys (Ernst and McBreen, 1991). Many populations have been declining for decades because of habitat fragmentation, road mortality, and disease (Hall et al., 1999; Stickel, 1978; Nazdrowicz et al., 2008; Roe et al., 2023). Consequently, Eastern Box Turtles are of conservation concern throughout much of their range. Having a better understanding of the behaviors of box turtles, such as how they utilize their environment and thermoregulate, allows biologists to create more effective conservation plans.

Two personality types of Eastern Box Turtles are currently recognized: bold and not bold (Kashon and Carlson, 2018), which are usually determined by how quickly turtles emerge from their closed shell after disturbance. Boldness has been shown to affect movement rate, home range size, date of emergence from brumation, and thermoregulation (Kashon and Carlson, 2018; Roe et al., 2023). Bold turtles in central Indiana have been found to have higher body temperatures than less bold turtles (Kashon and Carlson, 2018; Pich et al., 2019). My study of a middle Tennessee population of Eastern Box Turtles also shows that bold turtles have significantly higher body temperatures than less bold turtles, which adds to the growing body of evidence that personality can affect body temperature in box turtles (Kashon and Carlson, 2018; Pich et al., 2019). Warren (2022), who studied the same population as in this study, found that the turtles with higher body temperatures had higher body conditions and more shell damage, which he suggested was linked to higher boldness levels. My data supports his assumption about body temperatures, as I found bold turtles, regardless of sex, maintain higher body temperatures than less bold turtles (Figure 5). In examining the smooths of the bold turtles in figure 7, bold turtles had sharper high peaks during the day and sharper low peaks at night compared to less bold turtle smooths. One explanation for this is that these turtles are more willing to be exposed during the day to reach increased basking temperatures and are also willing to be more exposed overnight despite reaching lower body temperatures. Less bold turtles had flatter temperature smooths, but at cooler temperatures, which may suggest that they are more covered and hidden, but at the sacrifice of potentially never reaching an ideal temperature range. Because it is known that bold turtles from a southwestern North Carolina population had larger home ranges, emerged earlier from brumation, and experienced lower survival compared to non-bold individuals (Roe et al., 2023), it could be expected that these more conspicuous behaviors may be linked to bold turtles maintaining higher body temperatures. More conspicuous behaviors would make them unprotected more often but would also allow for more sunlight to reach them, increasing their body temperatures. With a better understanding of the traits that covary with personality, especially habitat utilization, we can more accurately predict the type of environments used and, thus, develop conservation plans and practices to aid in the conservation of Eastern Box Turtles.

In addition to personality type, behavior may be influenced by sex in box turtles, as shown for home range size, movement distances, utilization of developed habitats, and thermoregulation (Brisbin et al., 2008; Fredericksen, 2014; Kashon and Carlson, 2018; Warren, 2022). Fredericksen (2014) found that female box turtles had larger home ranges than males, and he attributed the difference in size of home range to preference of females to lay eggs in open sunlit areas. Males, and females outside the nesting season, typically avoided these open habitats. Brisbin et al. (2008) found that females were more likely than males to venture into developed areas and to cross streets, and that survival rates were lowered with increasing time spent in suburban neighborhoods. They predicted that the more common occurrence of females traveling into suburban areas with relatively lower survival rates may impact population dynamics in the future (Brisbin et al., 2008). Kashon and Carlson (2018) did not find an effect of sex on body temperatures of box turtles in central Indiana, but Warren (2022) noted that daytime temperatures differed between male and female turtles in middle Tennessee, where females exhibited higher temperatures. My data aligns with that of Warren (2022), as females in my study exhibit significantly higher temperatures than males (Figure 6). However, it is worth noting that I worked with the same population as Warren, and at least one turtle (HKW) was used in both studies, which may have affected my results. Maintaining relatively high body temperatures may be especially important in female box turtles to enhance digestion and the regeneration of fat reserves to support future reproductive bouts (e.g., oogenesis). Reported effects of sex on thermoregulation in box turtles have been inconsistent, perhaps in part because little research has been done on the topic. More research is needed on potential sex differences in behavior to have a better understanding of how different conservation techniques may affect males and females differently.

The interaction between sex and boldness of Eastern Box Turtles was also found to be significant. All combinations of sex and boldness (bold males, bold females, less bold males, and less bold females) were found to have significantly different average temperature smooths from one another (Figure 8). When comparing the two female smooths to the two male smooths in figure 8, males of different personality classifications had much greater variation than that of females of different personality classifications. Less bold males had a much lower smooth than all other sex and personality combinations, and bold males had the highest smooth compared to all other combinations of sex and boldness. In comparison, while bold and less bold females have significantly different smooths, there is much greater overlap in their smooths than in the smooths of bold and less bold males (Figure 8). This would suggest that personality in males has a larger effect on thermoregulation than it does in females. From these data, I would predict that less bold and bold females would move through their environment more similarly than males. I would also predict that less bold males are likely to move the least throughout their environment compared to all other groups, and that they are more willing to remain at cooler temperatures if it would mean being less conspicuous. Future studies that closely track precise movement of turtles in their environment as well as the frequency of movement would help build on this research to better understand the proximate mechanisms for these differences in thermoregulation.

Because Eastern Box Turtles are ectothermic, thermoregulatory behaviors are important when considering conservation concerns. Studies have been done to determine the preferred environment of box turtles and how they utilize their environment to thermoregulate (Adams et al., 1989; Fredericksen, 2014; Parlin et al., 2019). Eastern Box Turtles in southern Virginia spend most of the year in mature forest habitats; however, during May almost half the population occupies edge habitats (Fredericksen, 2014). Furthermore, turtle activity decreases as the habitat became hotter and drier, and activity also decreases as temperatures decrease in the fall months, suggesting the more moderate temperatures brought about the most activity from the turtles; rainfall also increases activity, especially after a long dry period (Fredericksen, 2014). Turtles in southwestern Ohio commonly selected evergreen and deciduous forests and herbaceous grasslands, even though those environments did not always have optimal temperatures (defined as the preferred body temperature at which performance of many physiological processes is at or near optimal) for the species, and movement was not correlated with the internal body temperature in turtles (Parlin et al., 2017). Thus, environmental temperatures are not the most important factor in habitat selection for box turtles (Parlin et al., 2017). A third study on thermoregulation in Eastern Box Turtles collected both laboratory and field data from southwestern Ohio on the potential dependence between temperature and locomotion (Adams et al., 1989). Box turtles increased locomotion speeds as temperature increased for both laboratory and field data, suggesting locomotion is strongly temperature dependent (Adams et al., 1989). Because of the importance of temperature on movement patterns in box turtles, the structure of a habitat is important to consider when designing conservation plans. An especially important choice likely to affect survivorship and energetics is that of an overwintering site.

Hibernation, often referred to as brumation in reptilian species, is important for box turtle survival in the cold winter months. A variety of factors on brumation in box turtles have been studied, including site preference, body temperatures, and conservation methods that may be implemented around brumation (Claussen et al., 1991; Currylow et al., 2013; Stickel, 1989; Walden, 2017). Stickel (1989), working in Maryland, found turtles rarely left their home range for hibernation. Sites selected for hibernation were depressions or forms in the forest floor that were filled with leaves, and the turtles would commonly dig into the side of the depression so that their back was fully buried under soil (Stickel, 1989). Turtles typically hibernated in approximately the same area for multiple years (Stickel, 1989); this has also been reported for a middle Tennessee population (Vannatta and Klukowski, 2015). Claussen et al. (1991), working with an Ohio population, found that turtles rarely buried themselves more than 4-5 cm under soil, that body temperatures of hibernating turtles were usually similar to the soil temperature, and some turtles that experienced body temperatures below 0° C successfully overwintered. Box turtles can tolerate freezing of > 50% of their body water and can remain frozen for at least 3 days without apparent injury (Costanzo and Claussen, 1990). Box turtles in central Indiana maintain an average body temperature of approximately 3.3° C while hibernating and the depth buried in the soil is dependent on the tree coverage of a hibernation site (Currylow et al., 2013). Turtles hibernating in open clearcut habitats encounter colder temperatures during hibernation but warmer temperatures when emerging from hibernation, compared to turtles that hibernate within a forested habitat (Currylow et al., 2013). Eastern Box Turtles select a variety of habitats for hibernation and can compensate for temperature differences in different habitats by changing the depth at which they hibernate. Monitoring the hibernation habits of box turtles in managed field environments allows for the determination of ideal times for mowing and maintenance of these habitats with minimal disturbance of box turtles, as they would be underground (Walden, 2017). Our understanding of these behaviors involved in brumation and how personality may influence them will allow for the implementation of less harmful land management practices, helping to protect the species.

Upon examining the brumation sites of eighteen Eastern Box Turtles across two winters in this study, neither sex nor personality had any significant effect on brumation site microhabitat selection. The turtles in this study selected brumation sites with a wide range of characteristics (see Table 5), however, those differences were not influenced by sex, personality, or their interaction. While these factors have no effect on microhabitat selection, it is still useful to conservationists to know that these factors have no effect, as it supports the microhabitat selection being a more random process based on the individual turtle, rather than sex or personality type. My data also provides a potentially useful description of the characteristics of the brumation sites chosen. For example, no box turtles chose sites that were fully exposed or fully covered (range of sky exposure: 35-85%). Currylow et al. (2013) found that depth of the brumating turtles in Indiana was dependent on tree coverage, so the tree coverage values may have influenced the depth at which the turtles were buried in middle Tennessee as well. All turtles were found in forms covered in leaves similar to what Stickel (1989) found in turtles in Maryland, but depths of the forms were very shallow, similar to what Claussen et al. (1991) found in Ohio. This suggests that in areas like middle Tennessee, where winters are generally mild, turtles can brumate safely at such shallow depths with little risk. Given this data on turtle brumation depth and tree coverage, researchers can predict brumation habits of box turtles in different climates when generating conservation plans.

Alongside thermoregulatory behaviors, I also examined the dates that the turtles entered brumation. No significance was found for sex, boldness, or their interaction on brumation start date. The earliest a turtle entered brumation was mid-October and the latest was mid-December, with the majority starting in mid- to late-November (Table 2). Claussen et al. (1991) found that turtles in Ohio typically enter brumation in mid-October to mid-November. Similarly, Currylow et al. (2013) found box turtles in Indiana typically enter brumation in mid- to late-October. One explanation for my turtles' delayed brumation start could be that, because middle Tennessee has more mild winters than Ohio and Indiana, the turtles tend to wait longer to enter brumation. Further research comparing different climates to the average start of brumation in box turtles would be needed to confirm this explanation.

CONCLUSION

This research gives new insight into the behaviors of Eastern Box Turtles during the fall and winter months and how factors such as sex and personality type affect the way these animals behave. Bold turtles were found to maintain a warmer body temperature than their less bold counterparts. Boldness had an especially large influence on thermoregulatory behaviors in male box turtles. While sex and boldness have a significant effect on thermoregulation, no effect of sex or boldness was apparent on brumation start date or brumation site microhabitat selection. Overall, this research contributes to our growing knowledge on Eastern Box Turtles and may help inform management practices to aid in their conservation.

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