Testing for Associations Between Boldness and Thermoregulation in the Eastern Box Turtle, *Terrapene carolina carolina*

by
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Abstract

The study of animal personality in non-mammalian species is a relatively new and active area of focus in the field of animal behavior. Consequently, many factors and their mechanisms which influence personality are still being discovered. Previous studies have shown that box turtles exhibit consistent individual behavior and that their thermoregulatory ability may be connected to their personality. In this thesis, I attempted to determine whether higher shell temperatures were correlated with bolder personalities in the eastern box turtle (*Terrapene carolina carolina*). Behavioral and thermal data was collected from 15 turtles during the summer of 2020 in Rutherford County, TN. The turtles did demonstrate consistent individual differences and were classified as bold or shy. However, there was not a significant correlation between boldness and the turtles' shell temperatures. This could reflect that turtles in naturally warmer climates do not need to expend much time and energy for behavioral thermoregulation, and, therefore, that boldness, as a personality trait, is uncoupled from thermoregulatory behavior. If correct, in more southern populations, boldness may have fitness benefits other than enhanced thermoregulation and the presumed higher growth rates.

TABLE OF CONTENTS

List of Figures

List of Tables

List of Terms

Behavioral Syndrome – A correlation between different types of behavior

Boldness – The propensity to engage in risky versus cautious behavior

Carapace – Upper portion of the shell

IACUC – Institutional Animal Care and Use Committee

Mesic – A type of habitat characterized by moderate amounts of moisture

Personality – Individual differences in behavior that remain consistent over time and through varying circumstances

Pinch Gauge Dynamometer – A device used to measure pinch or grip strength based on the amount of force applied to two metal finger plates

Plastron – Lower portion of the shell

TWRA – Tennessee Wildlife Resources Agency

Introduction

Personality plays a crucial role in how an individual lives their life. It shapes everything from the way someone copes with stress to the relationships they form and keep over time. The impact of personality on human life is something that has been widely observed and studied. By comparison, the study of animal personality has had a slow start with early studies, focusing on mammalian species, dating back to the 1970s (Ogden, 2012). However, recent studies have shown that numerous non-mammalian species, including reptiles, possess personality differences and these may be vital in determining life history traits. Personality, or animal personality, is defined as individual differences in behavior that remain consistent over time and through varying circumstances (Ogden, 2012; O'Malley et al., 2019; Wilson et al., 2019). Many recent studies on animal behavior have begun to discover just how impactful personality can be in areas such as longevity, growth, and social dynamics (Stamps, 2007; McEvoy et al., 2015; Ward-Fear et al., 2018; Wilson et al., 2019).

For example, according to Stamps (2007), bolder individuals across several species (e.g., fish, cattle, and human infants) have a higher growth rate than their more timid counterparts. This is partially supported by research conducted by Kashon and Carlson (2018) who studied boldness in the eastern box turtle (*Terrapene carolina carolina)*. They define boldness as, "the propensity to engage in risky versus cautious behavior" which, in box turtles, exhibits itself as biting, being less likely to retract into their shell or reopening their closed shell soon after closing it, and moving from a location relatively quickly after a threatening encounter. One of the reasons Kashon and Carlson (2018) proposed for the correlation between boldness and a higher growth rate, at least in box turtles, is that bolder individuals spend more time basking in the sun and, therefore, maintain a higher body temperature.

Kashon and Carlson (2018) found that boldness in box turtles was positively correlated with shell temperature (and therefore higher metabolic rates) which would presumably lead to increased growth and reproductive rates. These results are supported by several other studies which also suggest that thermoregulation is related to sex determination, clutch size, energy acquisition, and locomotion (Besson and Cree, 2009; Dubois et al., 2009; Rowe et at., 2017). This relationship is expected because turtles are ectothermic. Thermoregulation may play a key part in turtle ecology even during embryonic development. Du et al. (2011) found that Chinese soft-shelled turtle (*Pelodiscus sinensis*) embryos had the ability to move towards warmer temperatures in the egg. They hypothesized that this could increase a turtle's fitness by enhancing their development and potentially decreasing the incubation period which would lead to reduced risk from nest predation.

In adult eastern box turtles, however, Kashon and Carlson (2018) suggested that higher body temperatures were also linked to higher levels of injury. They hypothesized that the propensity of risky behavior in bolder box turtles could lead to more interactions with predators. Such injuries were most frequently observed on the turtles' shells which act as their primary line of defense during threatening situations. Box turtles have the ability to completely close their shell, with their head and limbs inside, due to the plastron (bottom part of the shell) being hinged (Bramble, 1974). Complete shell closure makes it nearly impossible for most predators to reach the softer, more vulnerable parts of the turtle. Though this ability is vital to box turtle survival, little research has been done regarding individual differences in shell closure behavior (Preston et al., 2020). In addition to personality differences, shell closure behavior may vary with a turtle's size or age.

The goals of this research project are to determine whether adult eastern box turtles exhibit consistent, repeatable individual differences in boldness (i.e., personality) and whether there is a correlation between boldness and body temperatures in a middle Tennessee population of the eastern box turtle. Kashon and Carlson (2018) conducted their study of the eastern box turtles in central Indiana – whether their findings are repeatable in a more southern population which has a different thermal environment is unknown. Additionally, the effect of body size and age will be examined for potential influences on shell closure behavior. If the findings of Kashon and Carlson (2018) apply to box turtles in general, regardless of geographic location, there should be repeatable differences in boldness among individual turtles and a positive correlation between boldness and body temperatures in Middle Tennessee. My hypothesis is that higher body temperatures will be linked to shorter head emergence latencies and to the use of more active defense mechanisms such as biting, hissing, or urinating. I also predict more cautious turtles will exhibit a higher shell closure force. In studying the behavior of the eastern box turtle, I hope to better understand the consequences of different temperaments (bold vs. cautious) from an evolutionary perspective. I also hope to illuminate some of the factors that influence shell closure force which could have a significant impact on box turtles' antipredation success.

Methodology

Preparation

Before observations and data collection could begin, permits had to be obtained from the city of Murfreesboro Parks and Recreation Department, the Tennessee Wildlife Resources Agency, and the MTSU IACUC in order to study and interact with the box turtles. Thermochron iButton temperature loggers (DS1921G, Maxim Integrated, San Jose, CA), were programmed to record turtle and environmental temperature every 30 minutes, and were coated with Plasti Dip® (Blain, MN) to protect them from inclement weather. Such protective seals have been shown to have minimal effect on the temperature logged by the iButton (Roznik and Alford, 2012; Milanovich, 2017). In addition, the five radiotransmitters used to relocate the turtles had to be purchased or refurbished.

Initial Capture and Data Collection

Because of the five transmitter limitation, each had to be used multiple times. Three groups of five turtles were therefore captured and observed during the study. Box turtles were hand captured from Nickajack Trace Wetlands (Murfreesboro, TN) during the periods of June 2-5, June 15-24, and July 6-13, 2020. The date, time, GPS coordinates, weather conditions, and the microhabitat where the turtle was located were all noted at the time of capture.

Each turtle was then checked to see if it had any identifying file marks on the edge of its carapace (the hard, top portion of the shell) which corresponded to a three-letter naming system in which each marked scute represented a specific letter of the alphabet. If they were marked, the corresponding identification (ID) was noted. If not, an unused ID was chosen for it and it was marked accordingly using a triangular file. The sex was determined from the turtle's eye color and plastron shape. Males have concave plastrons with thicker tail bases and bright orange or red colored eyes while females have flat or convex plastrons with dark red or brown eyes (Lenninger, 2002). Body mass was measured to the nearest 5 grams using a spring scale, and calipers were used to measure carapace length, carapace width, and maximum shell height to the nearest 1 millimeter. Relative age was estimated by counting the annuli (growth rings) on the turtle's carapace (Wilson et al., 2003).

After recording the body measurements, the iButton and radiotransmitter were attached to the posterior corners of the carapace using epoxy adhesive. Each turtle was kept in a plastic container overnight so the epoxy had enough time to fully harden. The container and all measurement tools were disinfected with a 5% bleach solution between turtles. Each turtle was then released the following day at the same location it was found to conduct its first behavioral assay.

Tracking and Behavioral Assays

During the initial behavioral assay, each turtle was handled the minimum amount to stimulate it to completely close up into its shell before being set down. This typically took less than 60 seconds. Each subject was then observed for up to 10 minutes from a distance of at least 7-10 meters or as far away as possible while still having it in view. Some observation periods ended early if the turtle fully opened its shell and moved over 1 meter away relatively quickly. While observing the turtle, I noted the time it took for the head (specifically the eye) to extend beyond the perimeter of the shell, for the head (entire skull) to fully emerge, for the front legs (ankle joint) to extend beyond the perimeter of the shell, for the turtle to move 2 body lengths, and for the turtle to move 1 meter.

During subsequent assays, the turtle had to be located again using radiotelemetry. Once the turtle was found, I again recorded the date, time, weather, and the GPS coordinates. Then, the turtle was handled for 3 minutes by repeatedly taking its body measurements with calipers before being set down on the ground. I then observed the turtle from approximately 10 meters for up to 10 minutes. If the turtle would not tightly close its shell after the 3 minutes of handling, then additional stimuli (e.g., gently poking its face or squeezing its legs) were applied until it closed up completely. Each turtle (with one exception) underwent 5 behavioral assays with 2-4 days between each test. One turtle went through only 4 behavioral assays because it crossed a fence into private property. This subject was very difficult to relocate even after contacting the owner of the property to get permission to cross the fence. Once the turtle was found, it was tested, but in order to prevent having to locate the turtle again, it was only tested four times.

Final Measurements

After the last behavioral assay, pictures were taken of the turtle's skin and shell. These photographs were taken in the shade with a digital camera, and each photograph included a color standard to control for differences in ambient lighting. The turtles' body mass, shell pinch force, and injury score were then recorded. The pinch force was measured by putting a pinch gauge dynamometer (Baseline® 30 lb Pinch Gauge, Catalog no. 12-0200, Fabrication Enterprises Inc. White Plains, NY) between the turtle's anterior carapace and plastron. The turtle would try to close its shell, and the pinch gauge measured how many kg of force it generated. Unfortunately, I did not have the pinch gauge for the first set of turtles, so this measurement was only collected for 9 of the turtles. The injury score was assessed by observing injuries to the carapace and plastron which were classified on a scale of 0-24 using the same system as Saumure et al. (2007) and Kashon and Carlson (2018). The carapace and plastron were divided into four quadrants which were each scored from 0-3 based on the most severe injury it contained. A score of 0 meant there were no visible injuries, 1 meant there was little damage to the scute layer, 2 meant there was a lot of damage to the scute or part of the shell border was missing, and 3 meant there was a lot of damage to the scute and underlying bone. The sum of these scores was then used as the total injury score. After all the necessary data was collected, the iButton and transmitter were removed by gently prying them off with a pocketknife and the turtles were released.

Environmental Stations

To facilitate interpretation of a turtle's thermoregulatory behavior, in addition to the turtle iButtons, two environmental stations with four iButtons each were set up at the field site. The two environmental stations were separated by approximately 200 meters. These four iButtons recorded temperatures at 10 cm under the soil surface, at the soil surface, 5 cm above the ground, and 1 m above the ground. The iButtons at the surface and above were covered with an aluminum tent in order to prevent the black plastidip coating from absorbing excess sunlight which would skew the temperature data. These stations were checked every 2-4 days to make sure they were properly functioning.

Data Analysis

The iButtons used in the project were calibrated in order to ensure their accuracy. This was done by sealing all the iButtons used in the study in a single plastic bag which was placed at room temperature for 24 hours and noting any outliers with consistently high or low temperature readings. Most of the iButtons reported temperatures within 0.3°C of each other, but there were two outliers among them, one with temperature readings about 0.3°C too low and the other with readings about 0.5°C too high. The data for these two iButtons were corrected by the appropriate factor – in any case, both of these iButtons were used at environmental station two which was not used in the analysis.

For the turtles, the temperature data used in the analysis started 4 hours after their release and ended at the last temperature recorded before their final behavioral test. Because a box turtle's body temperature is heavily dependent upon the environmental temperature, to estimate a box turtle's thermoregulatory behavior I corrected shell temperature by the reference environmental temperature. This was done by subtracting the temperature of the ground surface at environmental station 1 from each subject's shell temperature at the corresponding time points.

The surface temperature was chosen rather than, e.g., the temperate at 1 m high because surface temperature most closely matched shell temperatures and because this method was used by Kashon and Carlson (2018). I only included environmental station 1 because the two least accurate iButtons were located at station 2, but in general the results from the two environmental stations were very consistent. For each subject, I calculated the average temperature difference for the full study period, for daytime temperatures (9 AM-5 PM), and for nighttime temperatures (11 PM-5 AM). Average time for behavioral latencies (e.g., eye emergence, skull emergence, front leg emergence, moved two body lengths, moved one meter) were also calculated. Turtles were classified as shy or bold based on their eye emergence latencies. Those in the bold group had average latencies less than 35 seconds, and those in the shy group had average latencies greater than 60 seconds.

Statistical analysis of the data was completed using SigmaStat 4.0 (Systat Software, Inc., San Jose California USA, https://systatsoftware.com/products/sigmastat/). A Spearman Rank Order Correlation test was used to test for correlations between average eye emergence latency, temperature, injury score, pinch strength, carapace length, body mass, and estimated age. Independent samples t-tests were used to determine whether shy and bold turtles differed in behavioral latencies other than eye emergence, corrected shell temperature, pinch strength, or body size. Finally, Mann-Whitney Rank Sum tests were used to test whether shy and bold turtles differed in age or injury score.

Results

Based on my observations, eastern box turtles do demonstrate robust personalities. The majority of the box turtles (9 of 15 subjects: 1-7, 12 and 15) displayed very consistent responses to the standardized 3 minutes of handling disturbance across the five assays (Figure 1). In fact, one box turtle (subject 1) emerged from its shell immediately upon release in each of the five assays. Also, the subject with the longest (i.e., most shy) eye emergence latency (subject 15), was very consistent in its response. Bolder turtles were more likely to exhibit defensive behaviors such as urinating, which occurred twice in the turtle with the highest boldness score, and struggling or kicking during handling, which occurred a total of six times only among the turtles with the top four boldness scores. Turtles in the bold group also tended to be more reluctant to retreat completely into their shell during handling than those in the shy group. Those in the bold group resisted closing their shell fourteen times compared to the two times exhibited by those in the shy group.

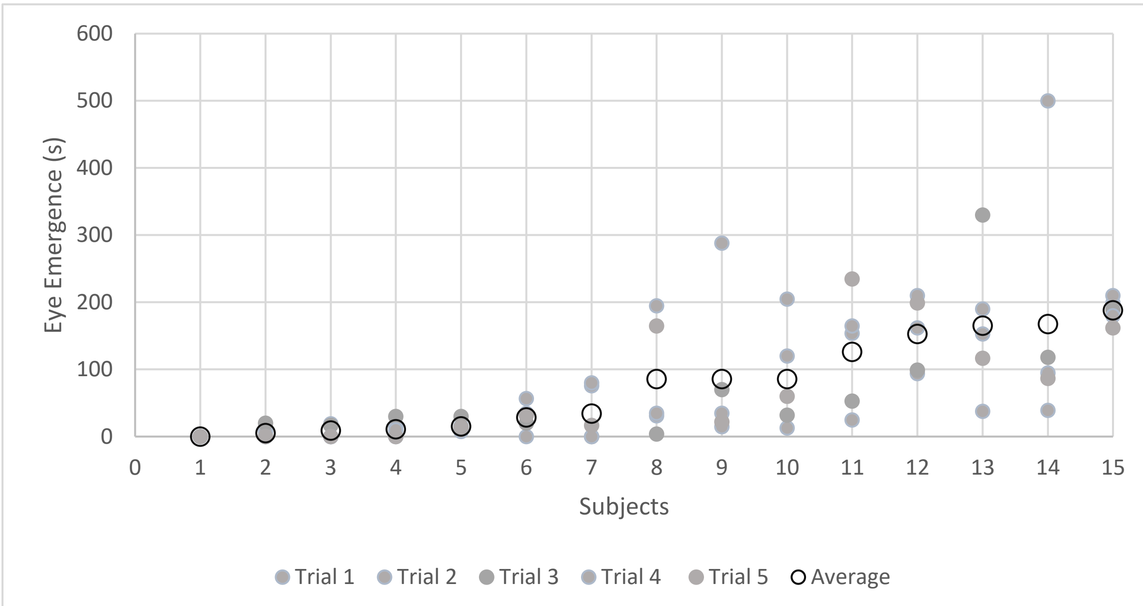

Figure 1. Latency (sec) for the eyes to emerge beyond the margin of the carapace after capture, 3 minutes of standardized handling, and subsequent release in eastern box turtles (*Terrapene c. carolina*). Each of the 15 turtles was tested in 5 assays separated by 2-4 days with the exception of subject 3 which was only tested in 4 assays. The open circles represent the average latency for each tested turtle which are arranged from left to right in order of increasing average latency. Turtles in which only 1-2 assays are visible indicates very little variability in responses (i.e., the eyes of subject number 1 emerged immediately upon release in all five assays). The first 7 turtles represent those in the 'bold' group, and the last 8 represent those in the 'shy' group.

Box turtles with short eye emergence latencies, categorized as bold, also had significantly shorter skull emergence latencies than turtles in the shy group (Independent Samples t-test, $T = -5.1$, $df = 13$, $P = 0.00022$). The same pattern was found for front leg emergence (T = -4.5, df = 13, P = 0.00057), two body lengths movement (T = -3.5, df = 13, P = 0.0038), and 1 meter movement latencies (T = -2.1, df = 13, P = 0.056), though 1 meter movement was not significant. All five behavioral latencies were also highly positively correlated with one another ($P < 0.015$ for all).

No significant correlation between a turtle's 24-hour shell temperature (corrected for ground surface temperature) and its latency for the eye to emerge was found (Spearman, Rs = -0.065, n = 15, P = 0.81; Figure 2). Similarly, there was not an association between eye emergence latency and either daytime shell temperature (R_s = -0.13, P = 0.63) or nighttime shell temperature (R_s = 0.4, P = 0.14; Table 1). Furthermore, when categorized into bold or shy groups, bold and shy turtles did not differ significantly in average 24-hour shell temperature (t-test, T = 0.28, df = 13, P = 0.79), or when the analysis was limited to just daytime temperature or just the nighttime temperature (P > 0.50 for both).

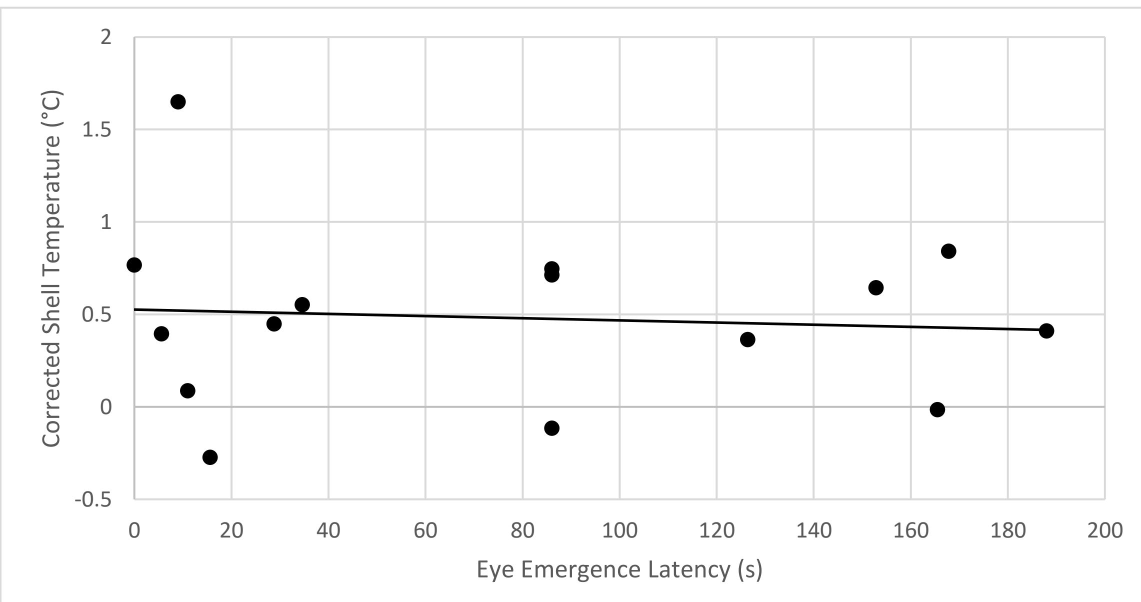

Figure 2. Average turtle 24-hour shell temperature, corrected by finding the difference from the ground surface temperature, compared to average eye emergence latencies.

Table 1. Associations between eastern box turtle average shell temperature and average behavioral latencies, injury score, and pinch strength. Average shell temperature is actually the difference between the carapace temperature and the temperature of the soil surface at the reference environmental station. Associations are shown for all the shell temperature points for each subject (24 hr Shell), for only the temperatures taken during the day from 9 AM until 5 PM (Daytime Shell), and for only the temperatures taken at night from 11 PM until 5 AM (Nighttime Shell). Fifteen turtles were used in each Spearman correlation coefficient test unless otherwise indicated. Behavioral latencies were averaged across all the assays. Significant associations ($P < 0.05$) are shown in bold.

	24 hr Shell	Daytime Shell	Nighttime Shell
Eye emergence	$R_s = -0.065$, $P = 0.81$	$R_s = -0.13$, $P = 0.63$	$R_s = 0.4$, $P = 0.14$
Skull emergence	$R_s = -0.11$, $P = 0.7$	$R_s = -0.15$, $P = 0.58$	$R_s = 0.21$, $P = 0.45$
Front leg emergence	$R_s = -0.018$, $P = 0.94$	$R_s = -0.079$, $P = 0.77$	$R_s = 0.29$, $P = 0.28$
Moved 2 body lengths	$R_s = 0.021$, $P = 0.93$	$R_s = -0.072$, $P = 0.79$	$R_s = 0.44$, $P = 0.098$
Moved 1 m	$R_s = 0.15$, $P = 0.58$	$R_s = -0.014$, $P = 0.95$	**$R_s = 0.75$, $P = 0.0009$**
Injury Score	$R_s = 0.12$, $P = 0.67$	$R_s = 0.13$, $P = 0.64$	$R_s = 0.08$, $P = 0.76$
Pinch Strength ($n = 9$)	$R_s = -0.067$, $P = 0.84$	$R_s = -0.2$, $P = 0.58$	**$R_s = 0.77$, $P = 0.012$**

No significant correlations were found between behavioral latencies and either injury score or pinch strength (Table 2). The closest to being significantly correlated was pinch strength which was positively correlated with eye emergence latencies – meaning that box turtles which took a relatively long time for their eyes to emerge tended to have higher pinch strengths. Similarly, bold and shy turtles did not differ in their injury score (Mann-Whitney, $T = 63.5$, $df = 13$, $P = 0.40$) or their pinch force (t-test, $T = -1.5$, $df = 13$, $P = 0.18$).

Table 2. Associations between shell injury score and pinch strength with average behavioral latencies in eastern box turtles.

	Injury Score (n = 15)	Pinch Strength (n = 9)
Eye emergence	R_s = -0.24, P = 0.38	R_s = 0.6, P = 0.08
Skull emergence	R_s = -0.28, P = 0.3	R_s = 0.2, P = 0.58
Front leg emergence	R_s = 0.36, P = 0.89	R_s = 0.32, P = 0.38
Moved 2 body lengths	R_s = 0.07, P = 0.8	R_s = 0.35, P = 0.33
Moved 1 m	R_s = 0.27, P = 0.91	R_s = 0.39, P = 0.29

Eye emergence did not have a significant correlation with either estimated age (Spearman, R_s = 0.1, n = 15, P = 0.72) or body mass (Spearman, R_s = -0.74, n = 15, P = 0.78). Neither was there a significant difference between shy and bold turtles in either estimated age (Mann-Whitney, T = -0.69, df = 13, P = 0.55) or body mass (t-test, T = 0.69, df = 13, P = 0.5). The average 24-hour shell temperature of box turtles was close to being significantly positively associated with body mass (Spearman, R_s = 0.48, n = 15, P = 0.066). Surprisingly, nighttime temperature had a significant positive correlation with both movement of 1 meter (R_s = 0.75, n = 15, P = 0.0009) and with pinch strength (R_s = 0.77, n = 9, P = 0.012; Table 1).

Discussion

The results of this study were quite different from that of Kashon and Carlson (2018) since no correlation was found between boldness and thermoregulation. This could be due to intraspecific differences between the more northern turtle population they studied in Indiana and the more southern population in Tennessee. Turtles in cooler climates may have a wider normal temperature range than those in warmer environments which would cause them to expend more time and energy basking in order to maintain an optimal body temperature (Rowe et al., 2017). Turtles farther south would not need to spend as much time basking since they should, theoretically, already be closer to the optimal temperature. Northern populations would be more likely to face temperatures low enough to impact their fitness, so they would benefit more from increased thermoregulatory behavior (Dubois et al., 2009).

In addition, factors such as water availability and altitude can impact basking frequency since they affect the thermal environment. For example, highland Tasmanian snow skink species (*Niveoscincus greeni* and *Niveoscincus microlepidotus*) spent more time basking than their lowland counterparts (*Niveoscincus ocellatus* and *Niveoscincus metallicus*) regardless of the available basking opportunities (Caldwell et al., 2017). Bodies of water in an animal's habitat can also influence their thermoregulatory behavior since they provide more stable temperatures than terrestrial habitats. Thus, during warmer seasons, these bodies of water will retain warmer temperatures and can then be used to regulate temperature and possibly increase metabolism at night when terrestrial habitats are cooler (Dubois et al., 2009; Rowe et at., 2017). My wetland field site was likely more mesic than the field site used by Kashon and Carlson (2018).

Boldness may also be associated with other personality traits in a behavioral syndrome. Pich et al. (2019) and Roth et al. (2020) found correlations between boldness and aggression in turtles. This could be a result of bolder turtles utilizing more active defensive measures such as biting or urinating against predators. Kashon and Carlson's (2018) study suggested that bolder turtles had a higher risk of predation, so these defensive mechanisms could be a means to offset the risks associated with their personality type. Higher activity rates, and potentially information gathering, are also connected to boldness (Pich et al., 2019). This raises the question of whether exploratory and/or curious behaviors might also be part of a behavioral syndrome with boldness. Future studies might attempt to answer this question.

The relationship between injury score and personality could also be explored further. Kashon and Carlson (2018) did not find a relationship between eye emergence and injury score, but they did discover that movement latency was almost significantly negatively correlated with injury score (i.e., bolder turtles tended to have more injuries). My results did not correspond with theirs, however. I did not find any correlations between injury score or any behavioral latencies. The closest relationship was between injury score and skull emergence latency which was still far from being correlated (R_s = -0.28, P = 0.3; Table 2).

In addition, the relationship between boldness and pinch strength should be examined more closely, as well as the potential link between a turtle's color, thermoregulatory behavior, and personality. In my research, eye emergence latency and pinch strength were close to having a significant correlation, and I believe that further analysis, with a larger sample size, would help clarify the relationship between these two characteristics. The color of a turtle has the potential to influence its solar heat gain and may be linked to its behavioral syndrome. While I took photographs of each subject in this study, the color analysis is being completed by another researcher. Future research might also examine the impact of physiological functions such as heart rate and stress hormone on behavioral phenotypes. Increased heart rate has been shown to be correlated with higher body temperatures and exercise, both of which have been demonstrated to be connected to boldness (Gatten, 1974; Pich et al., 2019). Continuing research in this field is important to help increase our understanding of personality and its impacts on life history traits, ecological processes, and wildlife conservation (Pich et al., 2019).

Appendix

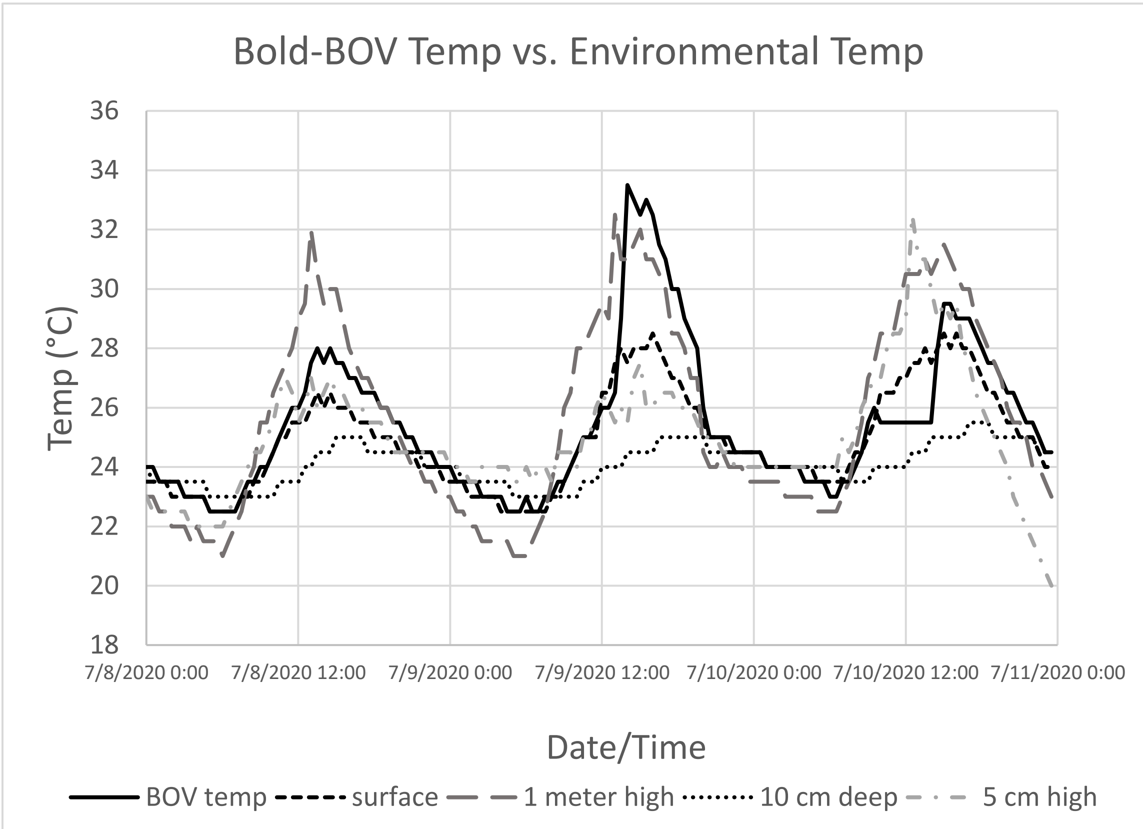

Figure 3. A three-day plot of a bold turtle and the environmental temperatures.

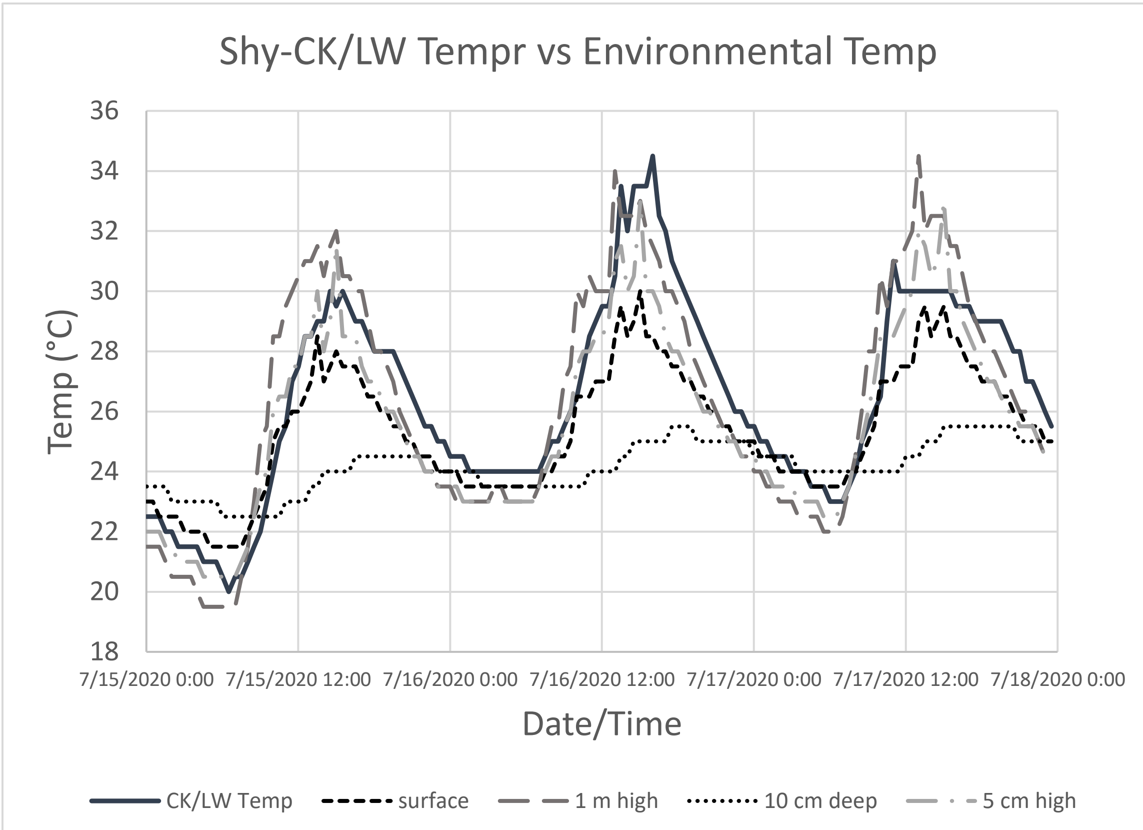

Figure 4. A three-day plot of a shy turtle and the environmental temperatures.

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