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Physiological and Behavioral Ecology of Juvenile Gopher Tortoises

A Thesis

Submitted to the Faculty

of

Drexel University

by

Thomas Andrew Radzio

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requirements for the degree

of

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Abstract

Physiological and Behavioral Ecology of Juvenile Gopher Tortoises

Thomas Andrew Radzio

Few terrestrial vertebrate ectotherms are strictly herbivorous, and those that are tend to inhabit tropical or warm desert environments, presumably reflecting thermal constraints on digestion of plant matter via fermentation. However, the imperiled gopher tortoise (*Gopherus polyphemus*) of the southeastern United States is solely herbivorous and often occupies shaded forests. Its ancestral environment is hypothesized to have consisted largely of warmer semi-open canopy longleaf pine (*Pinus palustris*) forests, but fire suppression and other factors have led to grand scale conversion of this highly biodiverse ecosystem to more shaded habitats. I investigated thermoregulatory strategies, thermal constraints on growth, and antipredator behavior of neonate, hatchling, and juvenile gopher tortoises in a longleaf pine forest of southwest Georgia. My findings indicate young gopher tortoises exploit most thermal opportunities available at burrows, basking increases growth rates, and low environmental temperatures likely constrain growth throughout the active season. Extensive basking begins at the neonate stage, where young exhibit a high-basking, rapid-growth strategy that exposes them to predation risk during thermoregulatory activity, but is presumably optimal overall. Although young bask extensively, their body temperatures may be particularly influenced by shade because they limit this activity to just in front of burrows. The field and laboratory data also suggest that tortoise body temperatures and thermosensitivity of growth are such that small changes in environmental temperature, including those that could be incurred by

increased shading or climate change can substantially impact growth rates and time spent at small body sizes, where individuals are most susceptible to predators. Although young tortoises can increase growth rates by basking, surface activity also increases exposure to predators. Individuals appear to manage predation risk by limiting most basking to burrow aprons and entrances, remaining vigilant, and quickly hiding belowground in response to potential predators. Simulated predator approaches on basking hatchlings and juveniles revealed very long flight initiation distances, which increased strongly with size/age, and apparent use of vibrations (aerial or ground) to detect and avoid danger, providing a possible ecological function for uniquely large otolith ear bones characteristic of this species. Consistent with the hypothesis that young exploit most thermal opportunities available at burrows to maximize growth, disturbed individuals hid for short durations, especially when using cool burrows. Similarly, surface activity during the hour following disturbance correlated negatively with burrow temperature. Tortoises raised in captivity during the first year of their lives for thermal physiology experiments also exhibited a high-basking, rapid-growth strategy and generally normal antipredator responses following hard release back into the field. Taken together, behavior, physiology, and environmental temperatures indicate warmer environments can reduce neonate–juvenile exposure to predators by increasing growth rates and, at least during certain times of the year, decreasing surface activity.

CHAPTER 1: INTRODUCTION

Understanding population responses to environmental variables is a central goal of ecology and conservation biology (Dunham 1993; Karieva et al. 1993). Longitudinal studies are invaluable, but such assessments often require multiple generations to deliver robust conclusions, which for long-lived species, may involve decades of field research (Balazs and Chaloupka 2004; Lovich et al. 2014). Alternatively, studying physiological and behavioral responses of individuals to environmental conditions can often provide rapid insights into important mechanisms influencing populations (Wikelski and Cooke 2006; Saba et al. 2012; Levy et al. 2015). This approach is becoming increasingly relevant as scientists grapple to understand how habitat alteration and climate change will influence populations, communities, and entire ecosystems (IPCC 2014).

In the southeastern United States, fire suppression and other land management practices have decimated the once dominant longleaf pine forest (*Pinus palustris*) ecosystem and often replaced it with closed-canopy forests that allow far less solar radiation to reach the ground (Van Lear et al. 2005; Watson and Gough 2012). Ectotherms adapted to life in semi-open canopy longleaf pine forests may face challenges associated with inhabiting cooler environments. The gopher tortoise (*Gopherus polyphemus*), a burrowing herbivore of the southeastern United States, is among a number of reptiles whose populations have experienced dramatic declines coincident with the conversion of longleaf pine forests to more shaded systems (Auffenberg and Franz 1982; Diemer 1986; Van Lear et al. 2005; U.S. Fish and Wildlife Service [USFWS] et al. 2013).

At highly shaded sites in Mississippi and Alabama, gopher tortoises grow very slowly and can require 20+ years to reach maturity (Aresco and Guyer 1999a; Holbrook et al. 2015). Slow growth may adversely impact tortoise populations by increasing time young individuals spend at small body sizes where they are highly vulnerable to a variety of predators, including snakes, mammals, birds, and invasive ants (Wilson 1991; Perez-Heydrich et al. 2012). In shaded environments, light levels at the forest floor may be insufficient to support high quality herbaceous forage, considered important for rapid growth (Aresco and Guyer 1999a; Mushinsky et al. 2003). Shade may further reduce growth rates by imposing thermal constraints on digestion (Avery et al. 1993; Aresco and Guyer 1999a), which can be particularly strong in herbivorous ectotherms (Zimmerman and Tracy 1989; van Marken Lichtenbelt 1992).

Many gopher tortoise conservation efforts prioritize protection and restoration of longleaf pine ecosystems. These actions can provide tortoises with high quality forage and thermal opportunities. However, understanding temperature influences on gopher tortoises and their populations remains important. Temperatures are forecast to increase in the future (IPCC 2014), and such information can improve predictions of tortoise population responses. Additionally, if temperature is important to growth, thermal heterogeneity may affect various other aspects of tortoise ecology, including burrow site selection, abandonment, and competition (Aresco and Guyer 1999b; Radzio et al. 2016; Chapter 5). Finally, our knowledge of tortoise ancestral habitats is limited. We may find that tortoises perform better in more open, warmer longleaf pine ecotypes (e.g., savannas) than in cooler ones. Similarly, we may find that warmer temperatures can compensate for reduced forage quality.

Behavior largely mediates relationships between environmental and body temperatures in ectotherms (Cowles and Bogert 1944). Many ectotherms behaviorally regulate body temperature such that physiological processes are enhanced (Lillywhite et al. 1973; Huey and Slatkin 1976; Bennett 1980; Angilletta 2001). Some ectotherms are precise thermoregulators and maintain relatively invariable body temperatures, despite thermal heterogeneity in their environment. On the other end of the spectrum are thermoconformers, organisms that allow their body temperatures to mirror those of their local surroundings. Theory predicts thermoregulatory strategies evolve to optimize benefits (e.g., increased energy assimilation) and costs (e.g., increased predation risk) of behavioral thermoregulation, which depend on many aspects of an organism's ecology (Huey 1974; Huey and Slatkin 1976). For example, traits such as cryptic coloration and specialized sensory capacities can reduce predation associated with thermoregulatory activity (Robert and Thompson 2007; Vitousek et al. 2007; Smith et al. 2016).

Two studies provide insights into the thermoregulatory or energetic strategy of neonate (0.0–0.5-year-olds) gopher tortoises. Bulter et al. (1995) placed small sticks in burrow entrances to monitor neonate activity and documented limited surface activity during the first six months of life. Similarly, Pike (2006) radio-tracked neonates and rarely observed foraging individuals. Both investigations suggest neonates may rely to some extent on residual egg yolk for sustenance until spring (Morafka et al. 2000). Although the activity observations suggest neonates do not attempt to maximize growth, Pike (2006) reported that individuals grew between their first fall and spring.

The thermoregulatory and growth strategies of hatchlings (0.5–1.0-year-olds) and small juveniles (> 1.0-year-olds) remain ambiguous. Similar to neonates, hatchlings and

small juveniles at a site in south Florida rarely emerged from burrows during summer and fall, on average only once every 3 days for 30 minutes (Pike and Grosse 2006).

Conversely, two other studies indicate small juveniles engage in substantial surface activity, dominated by basking directly in front of burrows (Wilson et al. 1994; Johnston 1996). Absence of temperature data make it difficult to evaluate differences in findings between Pike and Grosse (2006) and the other two investigations. Collectively, the data indicate small juveniles bask, perhaps to improve digestion (Troyer 1987; Zimmerman and Tracy 1989; van Marken Lichtenbelt 1992). However, the extent to which individuals exploit available thermal opportunities remains unclear.

Consistent across studies, young gopher tortoises limit basking activity primarily to the bare patch of soil located just in front of burrows, referred to as the “burrow apron” (Wilson et al. 1994; Butler et al. 1995; Johnston 1996; Pike and Grosse 2006; Pike 2006). This reluctance to spend time away from burrows may make it more difficult for individuals to thermoregulate effectively in highly shaded environments. Similarly, tortoise temperatures in burrows at night may be influenced by burrow exposure to direct solar radiation during the day. Given their habit of spending nearly all of their time at burrows (Wilson et al. 1994; Butler et al. 1995; Johnston 1996; Pike and Grosse 2006), immature gopher tortoises seem particularly likely to experience lower body temperatures in more shaded environments.

Similar to other herbivores, gopher tortoises rely on gut microbes to digest cellulose via fermentation, a process thought to require considerable time and high temperatures (Bjorndal 1987; Troyer 1987; Zimmerman and Tracy 1989; Tracy et al. 2005). Inability to maintain high body temperatures throughout the diel cycle may

constrain growth in ectothermic herbivores (Christian 1986; Tracy et al. 2005). Shapes of thermal performance curves may not only determine benefits of basking activity but also the extent to which small differences in burrow temperature, caused by shading or climate change (IPCC 2014), influence growth. If young typically exploit thermal opportunities when they are available, then we might expect individuals using cooler burrows to spend more time basking (Wilson 1991; Wilson et al. 1994). Thus, thermal constraints may not only cause young tortoises spend more time at small body sizes, but also spend more time thermoregulating outside burrows on a daily basis. Both mechanisms potentially increase interactions with predators and reduce recruitment to larger size classes.

I examined various aspects of the thermal ecology of immature gopher tortoises, including thermoregulatory strategy, thermal constraints on growth, and predator avoidance during thermoregulatory activity. Chapter 2 investigates thermoregulatory strategies and thermal constraints on growth in neonate, hatchling, and small juvenile gopher tortoises in a Georgia longleaf pine forest. A component of this work involved quantifying relationships between growth rate and temperature in the laboratory. Chapter 3 examines how behavior and temperature influence a tradeoff between thermoregulation and predator avoidance in hatchling and juvenile gopher tortoises. This work determined tortoise responses to simulated predator approaches and quantified temperature effects on hiding time and surface activity following disturbance. Chapters 4–5 relate to tortoise thermal ecology, but depart from thermoregulatory strategy and thermal constraints on growth. Chapter 4 examines thermosensitivity of hatchling righting response in the laboratory and combines findings with field observations to assess overheating risk

associated with overturning during activity. Chapter 5 describes encounters between immature tortoises at burrows, including aggressive interactions. Chapter 6 investigates behavior, growth and survival of tortoises raised in the laboratory for thermal physiology experiments following their release back into the field. As part of the work, I assessed the thermoregulatory and antipredator behaviors of the released animals.

CHAPTER 2: THERMOREGULATORY STRATEGIES AND THERMAL CONSTRAINTS ON GROWTH OF IMMATURE GOPHER TORTOISES INHABITING A LONGLEAF PINE FOREST

Introduction

Few terrestrial vertebrate ectotherms are strictly herbivorous, and those that are tend to inhabit tropical or warm desert environments (Zimmerman and Tracy 1989). This distribution may reflect reliance by herbivores on gut endosymbionts to digest most plant matter via fermentation, a process thought to require high temperatures (Troyer 1984, Bjorndal 1987, Zimmerman and Tracy 1989). Plant diets, which provide less energy than animal-based ones, may present special challenges for small ectotherms. For example, short thermal time constants can prevent small individuals from maintaining elevated body temperatures and digestive performance in refugia (Grigg et al. 1979, Christian 1986, Tracy et al. 2005). At the same time, thermal constraints on digestion may heighten demands on small individuals to thermoregulate behaviorally, which can increase exposure to predators (Vitt et al. 2005).

Consistent with hypothesized thermal constraints on energy assimilation in herbivorous ectotherms (Zimmerman and Tracy 1989), most North American tortoises inhabit deserts or open grasslands. However, the imperiled gopher tortoise (*Gopherus polyphemus*) of the southeastern United States often occupies shaded forests (Auffenberg and Franz 1982, Diemer 1986, U.S. Fish and Wildlife Service (USFWS) et al. 2013). It is hypothesized that its ancestral habitat consisted largely of warmer semi-open canopy longleaf pine (*Pinus palustris*) forests, but fire suppression and other factors, have led to grand scale conversion of this highly biodiverse ecosystem to more shaded environments

(Diemer 1986, Guyer and Hermann 1997, Van Lear et al. 2005, Watson and Gough 2012). Today, old-growth longleaf pine forest is among the most endangered ecosystems in North America (Noss et al. 1995).

Although causal data are lacking, reduced forage quality and temperature associated with habitat alteration may constrain growth rates of juvenile gopher tortoises in shaded environments (Aresco and Guyer 1999a, Holbrook et al. 2015). Low growth may adversely impact tortoise populations by extending time that individuals spend at small body sizes where they are highly vulnerable to predators (Wilson 1991, Perez-Heydrich et al. 2012). Annual survival of small juvenile gopher tortoises remains below 0.5 during the first several years of life (Wilson 1991, Perez-Heydrich et al. 2012), indicating that if individuals spend only one extra year at small size (< 100 mm), the number of animals recruiting into larger size classes can be substantially reduced.

Perhaps reflecting predation risk associated with thermoregulation, young gopher tortoises limit most basking activity to the bare patch of soil located immediately in front of burrows, referred to as the “burrow apron” (Wilson et al. 1994, Johnston 1996). This reluctance to bask away from burrows may make it more difficult for individuals to thermoregulate effectively in highly shaded environments. Similarly, tortoise temperatures at night track burrow temperatures (Johnston 1996), which may be influenced by daytime exposure to direct solar radiation. Given their habit of spending nearly all of their time in or near burrows (Wilson et al. 1994, Butler et al. 1995, Johnston 1996, Pike and Grosse 2006), immature gopher tortoises seem particularly likely to experience lower body temperatures in more shaded environments.

Studying thermoregulatory behavior and thermal constraints on growth in longleaf pine forests can provide insights into how young gopher tortoises perform in an environment that may closely resemble ancestral conditions (Diemer 1986, Guyer and Hermann 1997) and in other contemporary environments. If temperature and physiological thermosensitivity (Christian 1986, Zimmerman and Tracy 1989, Tracy et al. 2005) combine to constrain tortoise growth throughout a substantial portion of the active season in longleaf pine forests, we might predict greater thermal constraints in more-shaded systems (Aresco and Guyer 1999a) and weaker ones in warmer environments. Longleaf pine ecosystems are highly variable in canopy cover, and include savanna and sandhill ecotypes that may offer tortoises warmer conditions, especially under prevailing climate change scenarios (IPCC 2014).

I investigated thermoregulatory strategies and potential thermal constraints on growth in neonate (0.0–0.5 year), hatchling (0.5–1.0 year), and small juvenile (1.0–4.0 year) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. In the laboratory, I determined hatchling preferred temperature and quantified food intake and growth at different temperatures. For neonates in the field, I quantified microhabitat use, environmental temperatures, and growth following emergence from natural nests. For hatchlings and juveniles in the field, I quantified microhabitat use, environmental temperatures, shell temperatures, and exploitation of thermal opportunities available on burrow aprons. I also investigated potential relationships between tortoise temperature and growth in the field.

Materials and Methods

Study Site

I conducted field studies on Arcadia Plantation (30°45' N, 84°0' W), located in Thomas County in southwest Georgia, USA. The site (957 ha) was dominated by mature (> 80 years) longleaf pine forest and included the Wade Tract, an 80-ha old-growth ecological preserve that has never been heavily cut and contains many old trees (> 200 years; Platt et al. 1988). Ground vegetation on both the Wade Tract and surrounding areas of Arcadia Plantation was dominated by wiregrass (*Aristida stricta*), oaks (*Quercus* spp.), and a diverse assemblage of other native plants. Soils at the site were a mix of sand and clay. The study area was managed using frequent prescribed fire ($\leq$ 2-year return intervals).

Laboratory Animals

In September and October 2013, I collected 60 hatchling gopher tortoises as they emerged from nests at the study site. Additional descriptions of hatchling collection are provided in Radzio et al. (2017; Appendix 2). I maintained hatchlings in 53 × 9 × 4-cm (L:W:H) rectangular, black plastic enclosures. Most of each enclosure (43-cm length) was covered by a wooden board, providing individuals a long, dark and relatively cool burrow. A small portion of the enclosure (10-cm length) was only covered by a metal screen, where hatchlings could bask under a suspended heat lamp. I provided hatchlings a restricted diet consisting primarily of organic chicory (*Cichorium* spp.), orchard grass (*Dactylis* spp.), and Zoo Med Natural Grassland Tortoise Food (Zoo Med Laboratories,

Inc., San Luis Obispo, California). I soaked individuals in a shallow pan containing water, usually for at least 15 minutes, at least once every four days, but generally more frequently. I occasionally dusted food with Rep-Cal calcium and Herptivite supplements (Rep-Cal Research Laboratories, Los Gatos, California, USA.).

Light and temperature regimes differed minimally between when hatchlings were initially maintained in a field laboratory at Tall Timbers Research Station (early September–early November 2013) and during the following seven months preceding the growth experiment when hatchlings were maintained in the laboratory at Drexel University (early November 2013–early June 2014). During the 7-month period directly preceding the experiment, hatchlings were kept on a 13:11 light:dark cycle with heat lamps over basking areas turned on from 1000–1700 hour to facilitate behavioral thermoregulation, in which hatchlings appeared to engage extensively. Five days per week, 150-W neodymium basking bulbs provided operative temperatures of $\geq 40^{\circ}\text{C}$ at the basking end of enclosures. Two days per week, 160-W Powersun UV mercury vapor bulbs (Zoo Med Laboratories, Inc., San Luis Obispo, California) provided lower operative temperatures and UVB radiation at the basking areas. When basking lamps were off, room air temperature remained between 22–26 $^{\circ}\text{C}$. Room air temperature rose when basking bulbs were on, and depending on location within the laboratory, temperatures at the cool end of burrows ranged between 24–30 $^{\circ}\text{C}$. Minor deviations from the above regime occurred on a small number of days when individuals were used in other experiments (e.g., Chapter 4).

Hatchling preferred temperature

From late March–late May 2014, I determined preferred body temperature of fed hatchlings in the laboratory by measuring cloacal temperatures of 48 individuals in their enclosures. The enclosures did not represent proper thermal gradients per se (Camacho and Rusch 2017). However, I used this approach because the animals were very well acclimated to this environment and appeared to engage in normal thermoregulatory behavior. When placed in novel environments, individuals exhibited a persistent tendency to attempt escape.

A 150-W neodymium basking bulb provided operative temperatures of $\geq 40^\circ\text{C}$ at the basking end of enclosures. IButton miniature temperature data loggers (Maxim Integrated Products, Sunnyvale, California, USA) measured temperatures at different locations inside the burrow portion of enclosures. Temperatures at cool ends of enclosures varied between $24\text{--}30^\circ\text{C}$. However, I only used measurements where temperature at the cool end of the enclosure was $\leq 27.5^\circ\text{C}$ ($n = 35$) to estimate preferred temperature. I present observations for animals whose enclosure temperatures were $> 27.5^\circ\text{C}$ at the cool end ($n = 13$) separately.

On days preceding observations, I fed individuals wetted Zoo Med Natural Grassland Tortoise Food and soaked them in a container of water. I also attached an iButton, programmed to record temperature at 1-min intervals, to the rear of the carapace using double-sided tape. IButton data were not used to estimate preferred temperature, but provided a qualitative assessment of each animal's movements in the gradient. On the following day, I measured each hatchling's cloacal temperature once at a randomly selected time between 1100–1500 hours. Measurements were made 14 mm inside the

cloaca using a thin copper–constantan thermocouple. To avoid injuring tortoises, the end of the thermocouple was thinly coated with rubber. Before measurements, I lubricated the thermocouple using a very small amount of petroleum jelly.

Temperature effects on hatchling food intake and growth

From late June–mid August 2014, I determined hatchling food intake and growth at five constant temperatures (23.9, 26.6, 29.6, 32.6, and 35.4 °C). I assigned 11–12 individuals to each temperature treatment. Individuals from the same clutch were not placed together in temperature treatments, but assignments were otherwise random. Mean carapace length at the start of the experiment was 62.1 ± 2.8 mm and did not differ among treatments (ANOVA, $F = 0.049$, $df = 4$, $p = 1.0$).

Hatchlings were maintained individually in 30×12 -cm plastic boxes placed inside large, well-lit (13:11, light:dark cycle), temperature-controlled incubators. Each plastic box contained an artificial burrow (large plastic cup cut in half) and an iButton, programmed to record temperature at hourly intervals. Plastic boxes were located at three levels within incubators, and were systematically rotated throughout all spaces. An artificial light suspended from the top of the incubator provided individuals UVB light when they rotated through the top shelf.

From 23 June to 8 July 2014, individuals were provided *ad libitum* a diet consisting primarily of orchard grass (*Dactylis* spp.), but did not appear to grow. Individuals crawled through their water dishes, and evaporative cooling presumably prevented hatchlings from being at experimental temperatures.

On 8 July 2014, I switched hatchlings to an ad libitum diet consisting entirely of fresh locally obtained, organic *Cichorium*. I also removed dishes and provided water by soaking hatchlings daily. I soaked hatchlings by placing them in small containers of water, warmed to treatment temperature, and allowed them to drink inside incubators for ~15 min. After soaking, I briefly removed individuals from incubators, blotted them with a paper towel, and placed them back inside their boxes. Due to concerns that individuals were growing too rapidly (up to 8 mm·month⁻¹), I only maintained 29.6, 32.6, 35.4 °C treatments for 31 days. I maintained 23.9 and 26.6 °C treatments for an additional 10 days.

Intake measurements

I determined unrestricted food intake for each hatchling on days 4, 8, and 21, relative to the diet switch. On the day before observations, I removed petioles and midribs from *Cichorium* leaves using scissors, blotted excess moisture with a paper towel, and placed ~10 g of weighed leaves into resealable plastic bags. On the following day, between 0500–0600 hours (before lights turned on at 0600 hours), I placed hatchlings in clean boxes containing pre-weighed *Cichorium*. I briefly checked tortoises throughout the day to ensure individuals always had ad libitum food and added food in a small number of instances. Twenty-four hours later, I removed boxes, collected remaining leaves into aluminum weighing boats, and dried them in an oven overnight at 60 °C. To facilitate calculations of ingested food (described below), I quantified the relationship between leaf wet and dry mass using three food bags prepared in the same manner as those provided to tortoises.

Growth measurements

For 29.6–35.4 °C treatments, I measured hatchling mass on 6 days, spaced at regular intervals, following the diet switch. For 23.9 and 26.6 °C treatments, which ran longer, I determined mass on 9 days. For all temperature treatments, I measured midline plastron length (MPL) on days -15, -10, 23, and 31, relative to the diet switch. For 23.9 and 26.6 °C treatments, which ran longer, I also measured MPL on day 41. Additional measurements of 32.6 °C animals indicated no growth between days -10 and -2. Animals were measured using electronic calipers and weighed using a calibrated electronic balance.

Neonate activity, environmental temperatures, and growth in the field

Between 3–24 September 2013, I collected 10 neonates from 5 field-incubated nests (Radzio et al. 2017; Appendix 2). In the laboratory, I epoxied a miniature radio transmitter (model: BD-2; Holohil Systems Ltd, Carp, Ontario, Canada) with a short, flexible trailing antenna to the rear vertebral scute of each individual. To avoid adversely affecting shell growth, I did not attach transmitters to, or apply epoxy across, multiple scutes. Transmitters and epoxy weighed $\leq 4\%$ of neonate mass. I released individuals at their nest sites within 0–2 days.

Between 4 September–18 October 2013, I used time-lapse video cameras (Plotwatcher Pro, Day 6 Outdoors, Columbus, Georgia, USA) to quantify neonate microhabitat use and activity patterns. Cameras were mounted atop wooden poles and programmed to continuously record (0.1–1.0 frames·second⁻¹) activity at burrow entrances, burrow aprons, and a small area around burrow aprons during daylight hours.

To minimize influencing tortoise activity, I typically avoided visiting video-recorded neonates during the day.

I also recorded operative temperatures on neonate burrow aprons and temperatures in other small tortoise burrows at the site. IButtons, attached to rubber tubing with zip ties and rubber dip (Plasti Dip International, Blaine, Minnesota, USA), recorded temperature at different depths (10–75 cm) inside 5–6 hatchling and small juvenile burrows at 10-minute intervals. Simple metal models (described in Radzio and O'Connor 2017; Chapter 3) recorded operative temperatures on neonate burrow aprons at 10-minute intervals. Briefly, neonate models consisted of thin-walled aluminum jars with aluminum lids, measuring 2.6×5.0 cm and painted gray using Rust-Oleum Painter's Touch 2x Ultra Cover flat gray primer (model number 249088). An iButton temperature logger, epoxied inside the center of the hollow models atop a small piece of plastic, recorded temperature. Between 20–23 October 2013, I recaptured neonates to remove transmitters and obtain growth measurements. Animals were measured using electronic calipers and weighed using a calibrated electronic balance.

Hatchling–juvenile activity, thermal exploitation, temperatures, and growth in the field

In April–September 2013, I captured hatchling (0.5–1.0-year-olds) and small juvenile (1.0–4.0-year-olds) gopher tortoises using bucket traps at burrow entrances or by hand away from burrows. I epoxied an iButton, programmed to record temperature at 5-minute intervals, to the rear vertebral scute of individuals. I also epoxied a miniature radio transmitter to either the right or left posterior costal scute. To avoid adversely affecting shell growth, I did not attach equipment to, or apply epoxy across, multiple

scutes. Prior to attachment, I coated equipment with a thin layer of brown rubber (Plasti Dip International, Blaine, Minnesota, USA) to provide waterproofing and camouflage. I released individuals at their burrows within 24 h of capture.

Starting about two weeks later, I used time lapse video cameras and temperature loggers to quantify tortoise activity patterns, microhabitat use, and temperature relations. I typically aimed to make these observations on six, even calendar days, with dates scheduled in advance without respect to weather. Equipment failure and other issues sometimes intervened to reduce the number of observation days.

Time-lapse video cameras were set up in the same manner as for neonates. To avoid influencing tortoise behavior, I did not visit tortoise areas on recording days. An iButton recorded temperature 10 cm inside burrows of video recorded tortoises at 10-minute intervals. This distance inside the burrow typically represents the warmest location within burrows during most times of day when tortoises are active (Radzio & O'Connor, unpublished data). The iButton was affixed with epoxy to the end of a thin wooden spike such that the two objects created a nail-like shape. I then pushed the wooden spike into the sidewall of the burrow until the iButton laid securely flat against it. Metal models recorded operative temperatures on burrow aprons of video recorded tortoises at 10-minute intervals. For 0.5–2.0-year-old tortoises, I used the neonate models described above. For 2.5–4.0-year-old tortoises, I used larger, 4.3×8.2 cm, metal models of similar design (Radzio and O'Connor 2017; Chapter 3). After making observations, I recaptured tortoises, typically using bucket traps set in front of burrow entrances, to remove equipment and collect measurements. Animals were measured using electronic calipers and weighed using a calibrated spring scale or electronic balance.

Data Analysis

To estimate daily food intake of individual hatchlings in laboratory temperature treatments, I converted wet mass of food provided to dry mass (wet to dry conversion factor = 0.1053) and subtracted dry mass of food recovered 24 hours later. To adjust for differences in tortoise mass, I divided daily food intake by the mass of the individual. I then calculated a mean value for each hatchling using data from three intake trials.

I did not measure hatchling MPL on the day of the diet switch from grass to *Cichorium* (day 0). However, given no change in hatchling MPL between days -10 and -2 for 33 °C animals (not measured in other treatments), I assumed MPL on day -10 to approximate MPL on day 0. I calculated daily MPL growth from day 0 to better reflect performance on the *Cichorium* diet.

For each hatchling, I calculated daily change in mass by regressing mass against day (linear least squares, mean $r^2 = 0.90 \pm 0.09$, minimum $r^2 = 0.59$, $n = 58$). I also inspected plots for indications of changes in growth rate over time, which were not evident. Given heterogeneity of variances, I used Kruskal-Wallis tests, followed by Bonferroni-adjusted Mann-Whitney U-tests ($\alpha = 0.005$), to analyze potential temperature effects on mass-adjusted daily food intake, daily change in MPL, and daily change in mass.

In this thesis, I limit analyses of neonate activity and temperature to observations from 11–18 October 2013. Although video observations began shortly after neonates emerged from nests in September, I was primarily interested in developing behavioral insights into neonate thermoregulatory and energetic strategy. In many earlier video observations, neonates spent considerable time each day excavating burrows. At those

times, they may have basked to improve digging ability. However, if tortoises foraged regularly and basked extensively after having already constructed deep burrows, I would interpret such behaviors as more consistent with thermoregulation to relax constraints on digestion.

I determined the degree to which free-ranging hatchlings and juveniles fitted with iButton miniature temperature loggers exploited opportunities to elevate body temperatures to ranges that promote rapid growth, as indicated by the laboratory experiment. I limited this analysis to days when I video recorded and did not visit individuals. For each tortoise, I identified shell temperature recordings that coincided to times when temperature 10 cm inside the individual's burrow (typically the warmest in burrows during the day) was $< 28^{\circ}\text{C}$, but operative temperature on its burrow apron was $> 33^{\circ}\text{C}$. I then calculated thermal exploitation as the percentage of those shell temperature recordings that were $> 29^{\circ}\text{C}$. I also calculated a second estimate of thermal exploitation as the percentage of shell temperature recordings that were $> 29^{\circ}\text{C}$ or corresponded to times when individuals were at the surface. I did this because tortoises often basked on burrow aprons or in burrow entrances with their iButtons in the shade, scenarios where shell temperatures are likely to substantially underestimate internal body temperatures. These analyses were conducted using 19 animals (96 tortoise days) for which complete data (video and shell, burrow and apron temperatures) were available. I also summarize the corresponding video observations of activity and microhabitat use for these 19 animals.

I performed Pearson partial correlations to investigate potential relationships between growth rate (CL and MPL) and mean shell temperature of immature tortoises

(0.5–4.0-year-old) fitted with iButtons and recaptured approximately 1 month later. I performed these analyses using initial size or both initial size and day of year as covariates. Statistical tests were two-tailed, and unless otherwise specified, were evaluated at $\alpha = 0.05$. I performed all statistical analyses in SPSS version 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 24.0. Armonk, NY: IBM Corp.).

Results

Hatchling preferred temperature in the laboratory

I obtained a cloacal temperature for 48 fed, well-hydrated hatchlings. However, iButton data revealed that for 13 individuals, temperature in the cool end of the enclosure (inside the burrow) exceeded 27.5 °C. For measurements where temperature at the cool end of the enclosure was ≤ 27.5 °C, cloacal temperatures averaged 33.0 ± 1.6 °C (range = 27.9–35.9 °C, $n = 35$; Figure 1D). Only 1 of 35 individuals exhibited cloacal temperature below 30 °C. For measurements when temperatures in the cool end of the gradient were 27.5–30.0 °C, cloacal temperatures averaged 33.1 ± 2.0 °C (range = 30.5–36.9 °C, $n = 13$).

Temperature effects on hatchling food intake and growth laboratory experiment

Temperature influenced mass-adjusted daily food intake (Kruskal-Wallis test, $H = 42.5$, $df = 4$, $p < 10^{-7}$; Figure 1A), change in MPL (Kruskal-Wallis test, $H = 39.2$, $df = 4$, $p < 10^{-7}$; Figure 1B), and change in mass (Kruskal-Wallis test, $H = 31.8$, $df = 4$, $p < 0.00001$; Figure 1C). For each variable, values were highest near T_{pref} , but did not differ statistically between 29.6 and 35.4 °C (Bonferroni-adjusted Mann-Whitney U-tests, $\alpha =$

0.005; Figure 1A–D). All variables increased steeply between 23.9 and 32.6 °C and between 26.6 and 32.6 °C (Bonferroni-adjusted Mann-Whitney U-tests, $\alpha = 0.005$; Figure 1A–C). Although Bonferroni-adjusted tests did not reveal a statistical difference, MPL growth was greater at 32.6 than at 29.6 °C when analyzed without adjusting for multiple comparisons (Mann-Whitney U-test, $U = 33.0$, $p = 0.02$)

Neonate activity, environmental temperatures, and growth in the field

Nine of 10 neonates started constructing a burrow within 3 days of release, and the tenth neonate started within 7 days. Neonates may have initiated burrows sooner as I did not track all neonates early and often. All burrows were dug within 29 m of nests. Neonates appeared to have constructed deep burrows by early October 2013.

From 11–18 October 2013, neonates ($n = 10$ tortoises, 74 tortoise days) were active above ground daily, typically between 0930–1600 h (Figure 2). During these times, maximum temperatures inside 5–6 hatchling and small juvenile burrows averaged 23.5 ± 1.4 °C (range 21.0–27.5 °C), but operative temperatures on neonate burrow aprons averaged 34.2 ± 6.5 °C (median = 33.5 °C, range 20.0–57.0 °C). Neonates spent 203 ± 61 minutes (mean of individual tortoise means across observation days) on burrow aprons or in burrow entrances (with at least part of their body outside the burrow) and 25 ± 12 minutes at the surface away from burrow aprons each day. Surface activity away from burrows was distributed across a narrower, warmer portion of the day than was apron and entrance activity (Figure 2).

Neonates recaptured 25–47 days following release ($n = 7$, 20–23 October 2013) exhibited significant increase in carapace length ($t = 3.13$, $df = 6$, $p = 0.02$) and mass ($t =$

3.95, $df = 6$, $p = 0.01$), but increase in MPL was not significantly different from zero ($t = 2.38$, $df = 6$, $p = 0.06$). Carapace length increased 1.9 ± 1.6 mm (0.06 mm·day⁻¹, range = -0.9–4.0 mm). Mass increased 3.3 ± 2.2 g (0.11 g·day⁻¹, range = 0.4–6.1 g). The non-significant increase in MPL was 1.1 ± 1.2 mm (0.03 mm·day⁻¹, range = -0.4–3.4 mm).

Hatchling and juvenile activity and thermal exploitation in the field

During late April–mid October 2013, video-recorded hatchlings and juveniles ($n = 19$, 96 tortoise days) engaged in surface activity daily, the only exceptions being some cool, rainy days. However, even on cool rainy days, tortoises sometimes briefly emerged to drink from temporary water accumulations. Typically, surface activity occurred between 0800–1800 h, with movements away from burrows distributed across a narrower, warmer portion of the day than apron and entrance activity (Figure 3). Tortoises spent 229 ± 81 min (mean of individual tortoise means across observation days) on burrow aprons or in burrow entrances (with at least a part of their body outside the burrow) and 27 ± 15 min at the surface away from burrow aprons each day (Figure 3).

Shell temperature (33.3 ± 1.2 °C, mean of individual tortoise means, $n = 19$) was greater than mean burrow temperature at 10 cm (27.9 ± 2.0 °C, paired-sample t-test, $t = 10.0$, $df = 18$, $p < 10^{-8}$), but lower than mean burrow apron operative temperature (47.0 ± 2.2 °C, paired-sample t-test, $t = 25.2$, $df = 18$, $p < 10^{-14}$) during times when burrow apron operative temperature exceeded 40 °C (Figure 4). For each individual, mean shell temperature was intermediate to mean burrow temperature and mean apron operative temperature (Figure 4).

Tortoises exhibited shell temperatures $> 29^{\circ}\text{C}$ during $81.0 \pm 12.4\%$ (mean of individual tortoise means) of the time when apron operative temperatures exceeded 33°C and burrow temperatures were below 28°C (Figures 5A, 6). Tortoises exhibited shell temperatures $> 29^{\circ}\text{C}$ or were at the surface during $91.6 \pm 9.1\%$ (mean of individual tortoise means) of the time when environmental temperatures met the above criteria (Figures 5B, 6).

Hatchling and juvenile shell temperatures in the field

I obtained iButton shell temperature observations for 26 tortoises (1 hatchling and 4 juveniles in 2012, 9 hatchlings and 12 juveniles in 2013; Figure 7). In July–September 2012, mean shell temperature was $27.8 \pm 2.5^{\circ}\text{C}$ (median = 27.2°C , $n = 5$ tortoises, 40,960 observations), with 15.2% of observations $\geq 30^{\circ}\text{C}$. In April–May 2013, mean shell temperature was $24.8 \pm 4.7^{\circ}\text{C}$ (median = 23.6°C , $n = 12$ individuals, 75,386 observations), with 16.5% of observations $\geq 30^{\circ}\text{C}$. In June–Aug 2013, mean shell temperature was $28.0 \pm 2.9^{\circ}\text{C}$ (median = 27.1°C , $n = 13$ individuals, 72,117 observations), with 21.6% of observations $\geq 30^{\circ}\text{C}$. In September–October 2013, mean shell temperature was $27.0 \pm 3.1^{\circ}\text{C}$ (median = 26.1°C , $n = 3$ individuals, 22,566 observations), with 16.4% of observations $\geq 30^{\circ}\text{C}$ (Figure 7).

Hatchling and juvenile shell temperature-growth relations in the field

Among animals fitted with iButtons and recaptured approximately one month (31.3 ± 1.5 days, range = 30–35 days) later ($n = 15$), carapace length growth correlated positively with mean shell temperature when initial carapace length was used as a

covariate (Pearson partial correlation, $r = 0.74$, $df = 12$, $p = 0.002$) and when both initial carapace length and day of year were used (Pearson partial correlation, $r = 0.77$, $df = 11$, $p = 0.002$; Figure 8). Similarly, MPL growth correlated positively with mean shell temperature when initial MPL was used as a covariate (Pearson partial correlation, $r = 0.81$, $df = 12$, $p = 0.001$) and when both initial MPL and day of year were used (Pearson partial correlation, $r = 0.71$, $df = 11$, $p = 0.007$; Figure 9). The relationship between growth and mean shell temperature in the field was very similar to the relationship between growth and constant temperature treatment measured in the laboratory (Figure 9).

Discussion

This study indicates young gopher tortoises exploit most thermal opportunities available at burrows to elevate body temperature, that basking increases growth rates, and strongly suggests that growth remains constrained by low environmental temperatures throughout the active season in a Georgia longleaf pine forest. Extensive basking begins at the neonate stage, where young exhibit a grow-fast strategy that presumably exposes individuals to predation risk during thermoregulatory activity, but is optimal overall (Huey and Slatkin 1976, Urban 2007). The field and laboratory data also imply that animal temperatures and thermosensitivity of growth are such that small changes in environmental temperature, including those that could be incurred by increased shading (Watson and Gough 2012) or climate warming (IPCC 2014) can substantially impact growth rates and time spent at small body sizes, where individuals are particularly vulnerable to predators (Wilson 1991, Perez-Heydrich et al. 2012).

Hatchlings and juveniles exploited 82–92% of thermal opportunities available on burrow aprons to elevate shell temperatures above those found belowground. When surface temperatures were high ($\geq 40\text{ }^{\circ}\text{C}$), hatchlings and juveniles exhibited a mean shell temperature of $33.3\text{ }^{\circ}\text{C}$, which essentially corresponded to the preferred temperature of fed hatchlings in the laboratory. Although I did not measure neonate temperatures, individuals basked several hours each day. During most times, it was not possible for hatchlings or juveniles to attain $33\text{ }^{\circ}\text{C}$ without basking. Such temperatures were available in burrows only during midday to afternoon hours of very hot summer days (Radzio and O'Connor 2017; Chapter 3). Neonates, observed in October, could not achieve such temperatures or maintain them for long periods without basking.

The hatchling laboratory experiment indicates strong correspondence between preferred temperature and the temperatures at which individuals grow most rapidly. However, the data also indicate that individuals with access to high quality food can grow at low temperature ($23.9\text{ }^{\circ}\text{C}$), albeit very slowly ($1.2 \pm 0.7\text{ mm} \cdot \text{month}^{-1}$, MPL). From 23.9 to $32.6\text{ }^{\circ}\text{C}$, growth increased linearly at a rate of $0.47\text{ mm} \cdot \text{month}^{-1} \cdot ^{\circ}\text{C}^{-1}$ and peaked at $5.3 \pm 1.2\text{ mm} \cdot \text{month}^{-1}$. Hatchlings achieved these growth rates on an ad libitum diet of *Cichorium*, occasionally supplemented with calcium powder, which is not necessarily far superior to forage available at high quality sites. Wild juvenile gopher tortoises feed selectively on legumes and other plants high in nitrogen (Mushinsky et al. 2003), and food quality at my site, well-managed mature and old-growth longleaf pine forest, was presumably excellent. There, free-ranging hatchlings with high mean shell temperatures ($26.7\text{--}28.3\text{ }^{\circ}\text{C}$) grew $2.4\text{--}4.4\text{ mm} \cdot \text{month}^{-1}$ (MPL, $n = 4$), or up to one-third of the annual growth rate (Radzio and O'Connor, unpublished data).

Although a strong positive relationship between growth and temperature is not surprising for an herbivore (Christian 1986, Troyer 1987, Zimmerman and Tracy 1989), caution must be applied when interpreting data from constant temperature treatments (Niehaus et al. 2012). A major caveat of the laboratory work is that potential thermosensitivity of appetite (Alexander et al. 2001; Chapter 4) could have reduced intake in low constant temperature treatments (23.9–29.6 °C) more than would occur via temperature effects on gut passage time alone (Waldschmidt et al. 1986, van Marken Lichtenbelt 1992), and thereby inflated thermal constraints on growth. Although this concern cannot be alleviated, low variation in food intake at 23.9 and 26.6 °C suggest appetite was not a major factor, and consistency between laboratory and field growth patterns support the hypothesis of strong thermal constraints on growth, operating across a narrow range of ecologically relevant temperatures in these small herbivores. At minimum, the laboratory experiment indicates just how strong the temperature effect may be.

Despite basking extensively and being near preferred temperature (33 °C) for many hours each day, individuals spent half their time during summer 2012 and 2013 at 23–27 °C, temperatures that did not promote rapid growth in the laboratory. These temperatures largely reflect thermal conditions in burrows at night and during the day when surface temperatures were insufficient to elevate body temperatures. Given that the laboratory data suggest growth increases steeply with temperature from 23.9–32.6 °C, we can hypothesize that even small changes in burrow temperature, where individuals spend most of their time, could substantially impact growth. Similarly, theoretical and empirical work indicate that elevated night temperatures can increase growth in herbivores

(Christian 1986, Tracy et al. 2005). Environmental factors influencing burrow temperatures could include shade, soil characteristics, and air temperature (IPCC 2014).

Gopher tortoise growth rates vary considerably within the northern portion of the species' range and may partially reflect shade-induced thermal constraints on growth. Juveniles at sites in Mississippi and Alabama, characterized by low forage quality and high shade, grow very slowly (~ 7 and $9 \text{ mm} \cdot \text{year}^{-1}$, respectively) and can require more than 20 years to reach maturity (Aresco and Guyer 1999a, Holbrook et al. 2015). Individuals grow more rapidly ($13.5 \text{ mm} \cdot \text{year}^{-1}$) within the old-growth area of my study site (Radzio and O'Connor, unpublished data), where the native ecosystem is maintained by prescribed fire. However, consistent with thermal constraints on tortoise energetics, the highest measured growth rates of any northern gopher tortoise population occur on St. Catherine's Island, located off the Georgia coast. At this barrier island site, there is very little canopy cover, and tortoises grow a remarkable $19 \text{ mm} \cdot \text{year}^{-1}$ and mature in under 10 years (Harris et al. 2015), essentially matching tortoises living in central Florida, where the activity season is longer (Mushinsky et al. 1994).

Two additional studies provide circumstantial support for the hypothesis that shade can constrain gopher tortoise energetics and vital rates. Diemer and Moore (1993) reported substantial increases in juvenile growth and female clutch size following a large-scale experimental clearcut in north Florida. In another study, Hathaway (2012) reported extraordinarily large clutch sizes for female gopher tortoises translocated to improved cow pastures in south Florida where trees were absent from most enclosures. Temperature was not mentioned as a possible causal factor for the patterns observed in either study. However, given that temperature apparently constrains growth of young

gopher tortoises throughout the active season at my study site, a well-managed longleaf pine forest, it seems reasonable that relaxation of thermal constraints may have contributed to the above described patterns in growth and fecundity.

Although young tortoises can increase growth rates by basking, surface activity presumably also increases exposure to predators. Individuals appear to manage predation risk by limiting most basking to burrow aprons and entrances, remaining vigilant, and quickly hiding belowground in response to potential predators (Radzio and O'Connor 2017; Chapters 3 & 6). Simulated predator approaches on basking hatchlings and juveniles reveal long flight initiation distances and apparent use of ground vibrations to detect and avoid danger (Radzio and O'Connor 2017; Chapters 3 & 6), possibly facilitated by uniquely hypertrophied otoliths and other specialized inner ear morphology (Bramble 1982, Bramble and Hutchison 2014). Consistent with the hypothesis that young exploit most thermal opportunities to maximize growth, disturbed individuals typically did not hide in burrows for long durations (Radzio and O'Connor 2017; Chapters 3 & 6).

This investigation raises additional questions associated with the thermal ecology of gopher tortoises. For example, future work might examine potential roles of thermal environment in burrow site selection and abandonment (Guyer and Hermann 1997, Aresco and Guyer 1999b), thermoregulatory activity within burrows at night (Johnston 1996) and its energetic consequences, temperature effects on basking activity and interactions with predators (Radzio and O'Connor 2017; Chapter 3), and potential climate change effects on tortoise vital rates and population persistence. This study also highlights challenges and opportunities associated with conservation and management of northern gopher tortoise populations, where thermal constraints may contribute to low

juvenile recruitment and aging populations (Epperson and Heise 2003, USFWS et al. 2013). In particular, reintroduction or relocation projects (Ashton and Burke 2007, Tuberville et al. 2008) may benefit by taking thermal environment into account when making management decisions for this imperiled species.

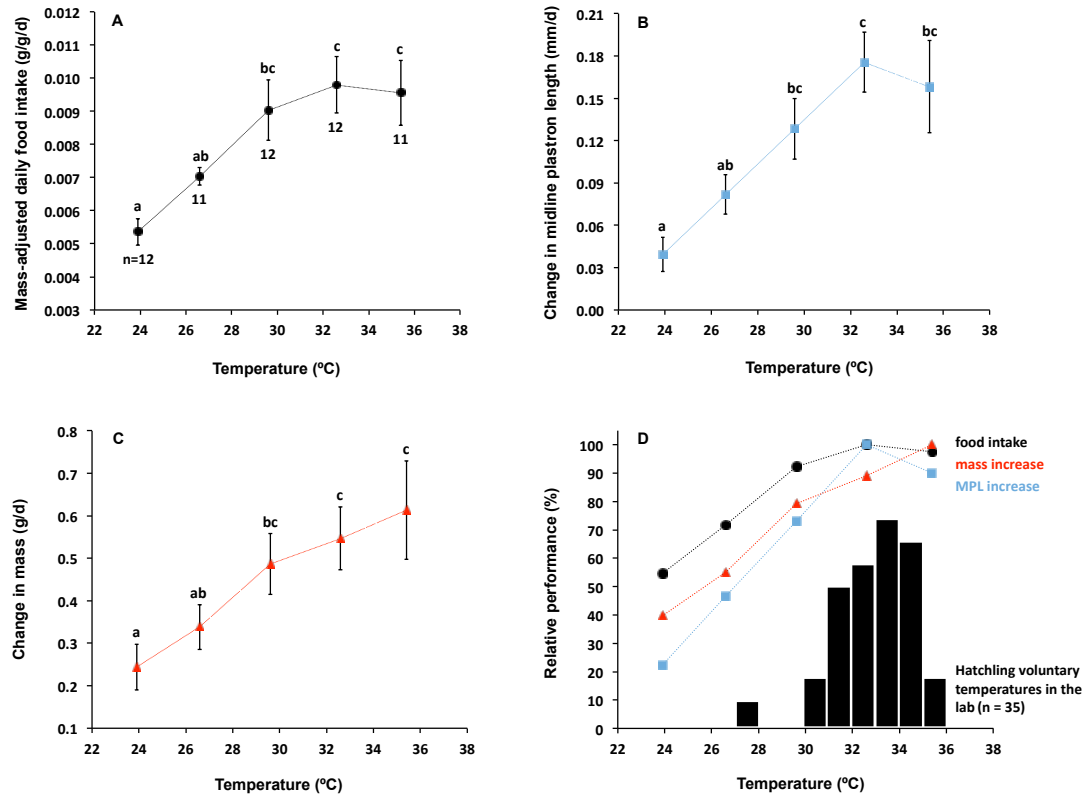


Figure 0-1. Hatchling (8–11-month-old) gopher tortoise food intake (A) and growth (B–C) under five constant temperatures in the laboratory when food was unlimited. Error bars are 95% confidence intervals of the mean. Relative performance for food intake and growth and the distribution (black bars) of hatchling voluntary temperatures in the laboratory when given access to 27.5–40+ °C (scale is relative, but not shown; D). See Discussion for limitations of constant temperature experiments.

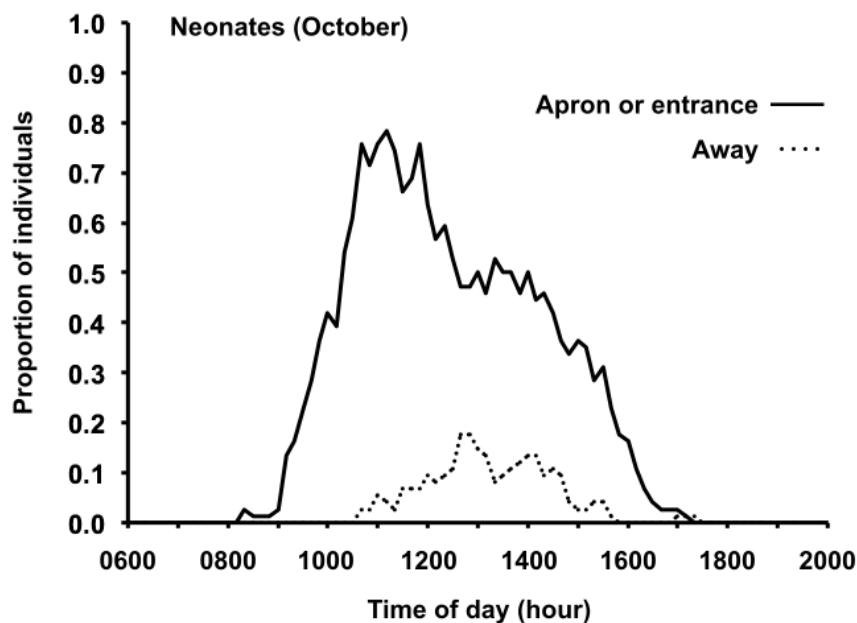


Figure 0-2. Microhabitat use patterns of video-recorded neonate (0.5–1.5-month-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. Solid line represents mean proportion of individuals ($n = 10$, 74 tortoise days) on burrow aprons or in burrow entrances (with at least part of their body outside the burrow). Dotted line represents mean proportion of individuals surface-active away from burrow aprons.

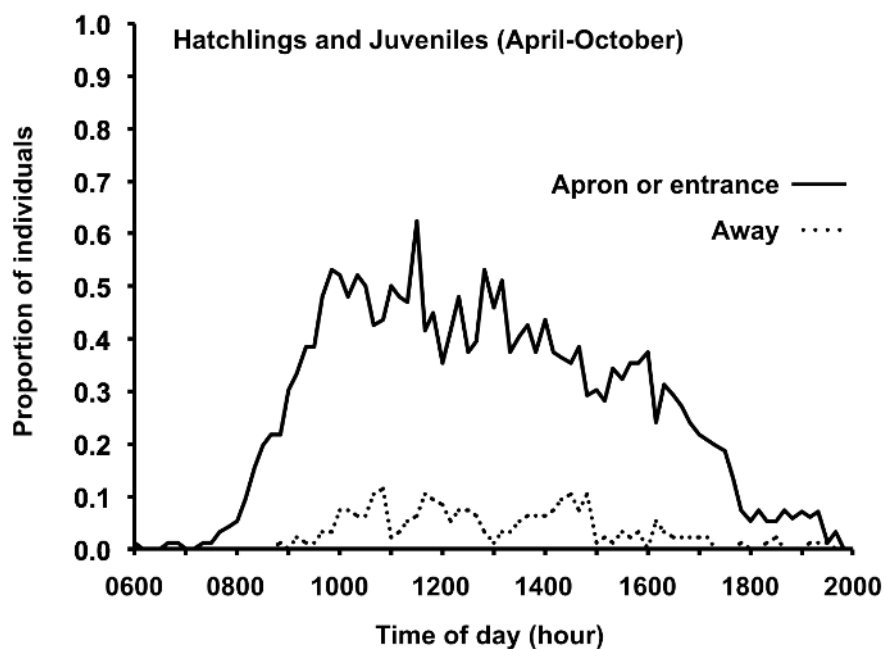


Figure 0-3. Microhabitat use patterns of video-recorded hatchling (0.5–1.0-year-old) and juvenile (1.0–4.0-year-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. Solid line represents mean proportion of individuals ($n = 19, 96$ tortoise days) on burrow aprons or in burrow entrances (with at least part of their body outside the burrow). Dotted line represents mean proportion of individuals surface-active away from burrow aprons.

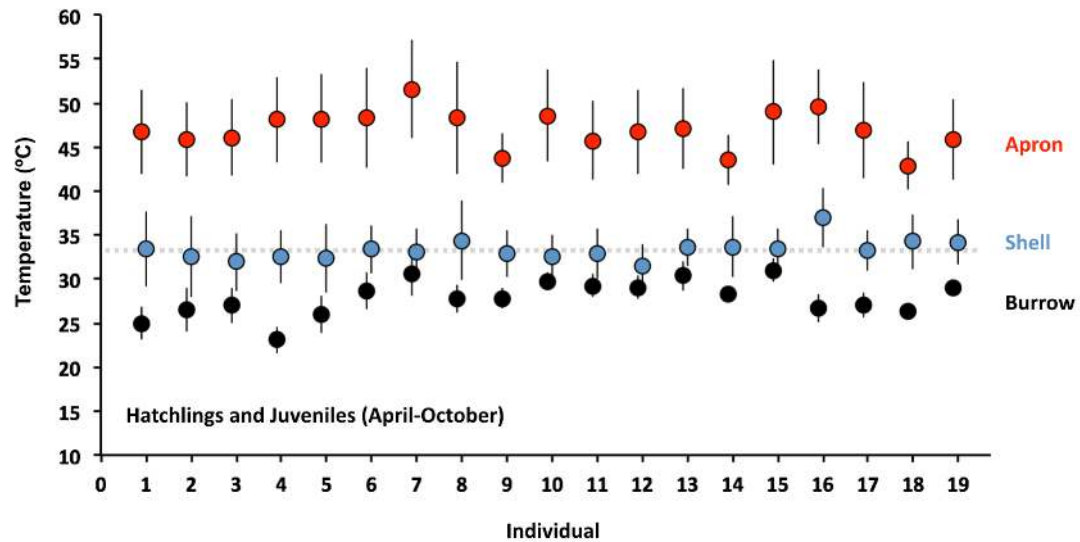


Figure 0-4. Tortoise shell (blue) and environmental temperature (burrow = black, burrow apron = red) relations when burrow apron operative temperatures were ≥ 40 °C, periods when it was possible for individuals to readily achieve T_{pref} by basking, for 19 hatchling (0.5–1.0-year-old) or juvenile (1.0–4.0-year-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia. Burrow temperatures are for 10 cm inside burrows, which typically represents the highest values available below ground during warmer times of day. Error bars are one standard deviation about the mean, and the dotted gray line represents T_{pref} of fed hatchlings (33.0 ± 1.6 °C). On average, observations span portions of 3.9 ± 1.5 days (range = 1–6) and include 93 ± 47 (range = 3–168) shell temperature measurements for each individual.

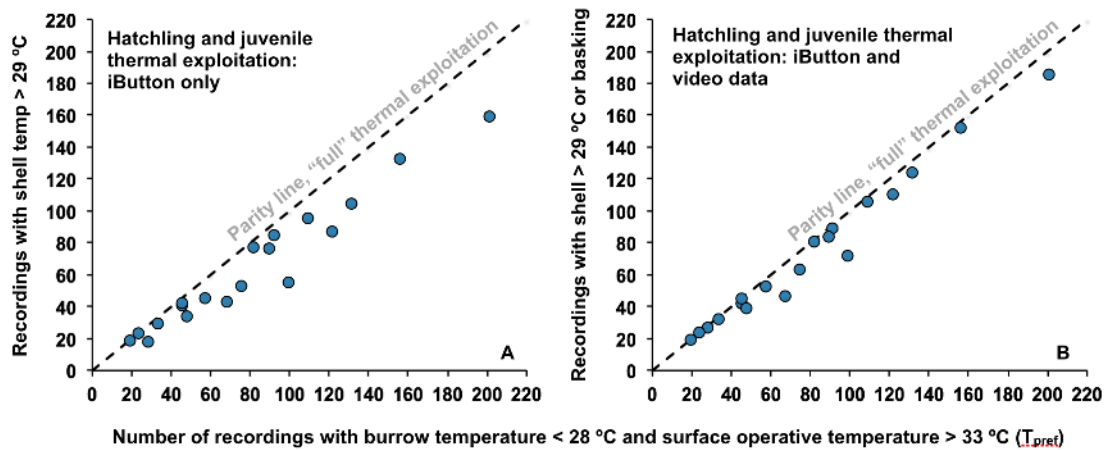


Figure 0-5. Measures of thermal exploitation for 19 hatchling (0.5–1.0-year-old) or juvenile (1.0–4.0-year-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. In both panels, the x-axis represents thermal opportunity, specifically, the number of recordings when it is possible for individuals to reach T_{pref} by basking on burrow aprons, but not by remaining inside burrows. The y-axis in the left panel represents number of thermal opportunities exploited by the individual, with exploitation defined as having a shell temperature > 29 °C. The y-axis in the right panel also represents the number of thermal opportunities exploited by the individual, but exploitation is defined as the as having a shell temperature > 29°C or being at the surface.

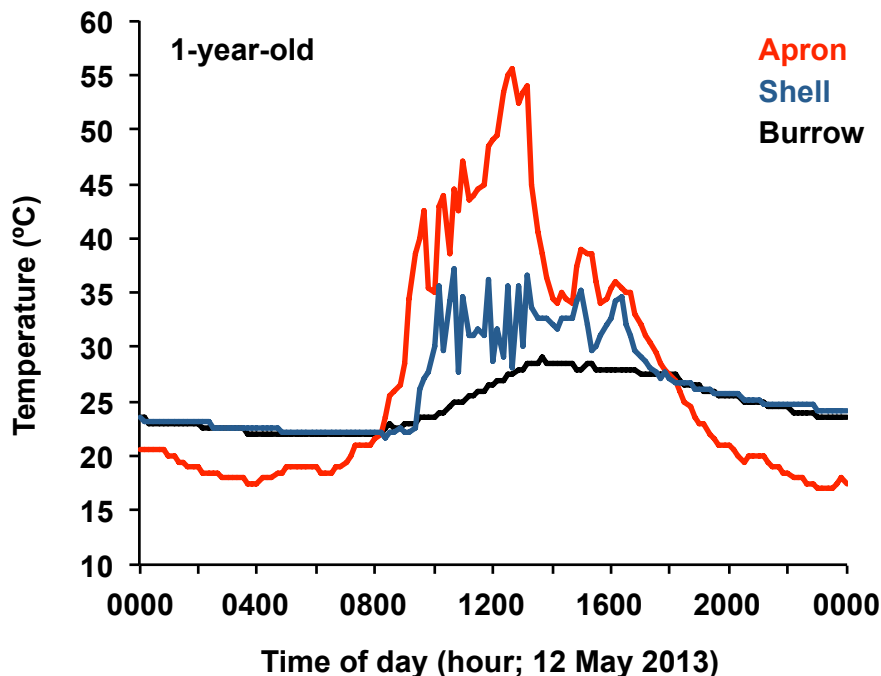


Figure 0-6. Example thermal profile for a 1-year-old gopher tortoise that exploited 76.5–94.1% of thermal opportunities available on its burrow apron on 12 May 2013 in a longleaf pine forest of southwest Georgia, USA. Burrow temperature (black) was recorded by an iButton secured 10 cm inside the burrow. Temperatures at this location typically match or exceed those found deeper in burrows during times of day when tortoises are surface-active. Burrow apron operative temperatures (red) were obtained using metal models containing iButtons. Shell temperatures were obtained by epoxying iButton miniature temperature loggers, thinly coated in brown rubber, to the rear vertebral scute of individuals.

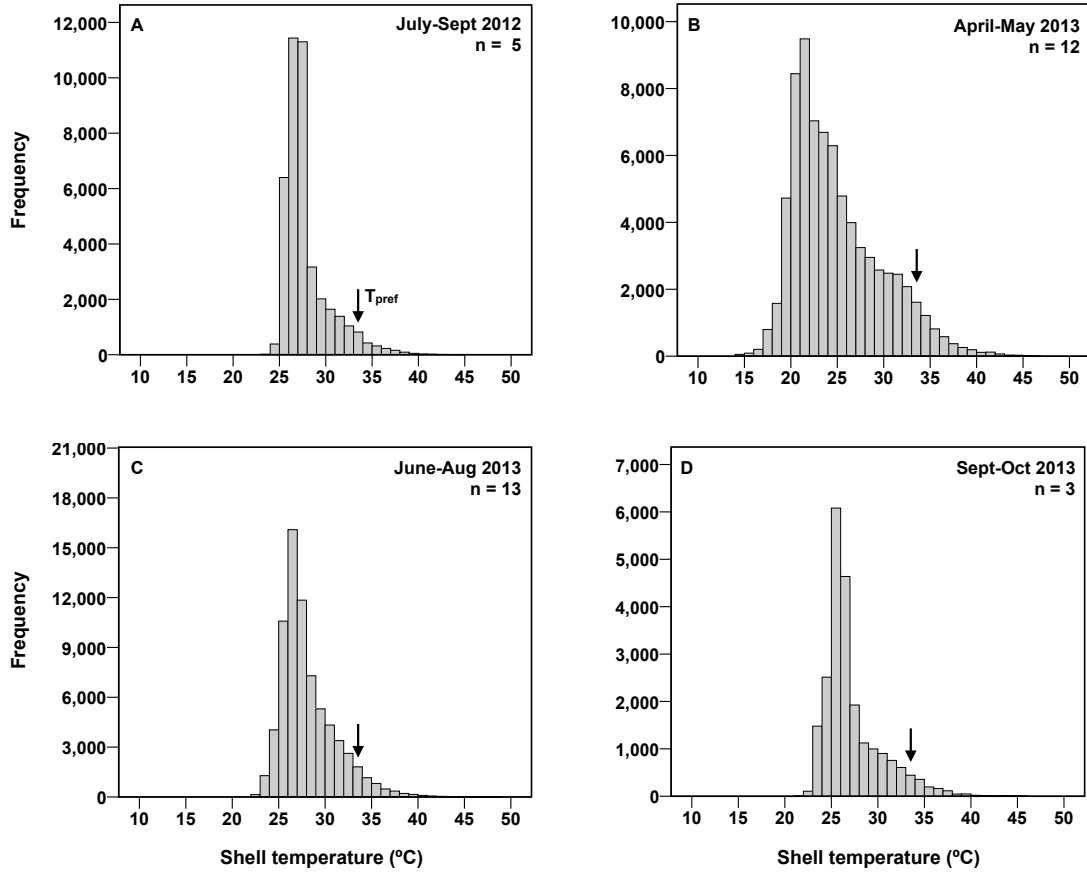


Figure 0-7. Active season shell temperatures of hatchling (0.5–1.0-year-old, $n = 10$) and juvenile (1.0–4.0-year-old, $n = 16$) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. Data were obtained by fitting free-ranging individuals with miniature iButton temperature data loggers. T_{pref} denotes preferred temperature of fed hatchlings measured in the laboratory (33.0 ± 1.6 °C). Despite exhibiting a strong tendency to exploit thermal opportunities, individuals could spend little time at T_{pref} , presumably owing to thermal constraints.

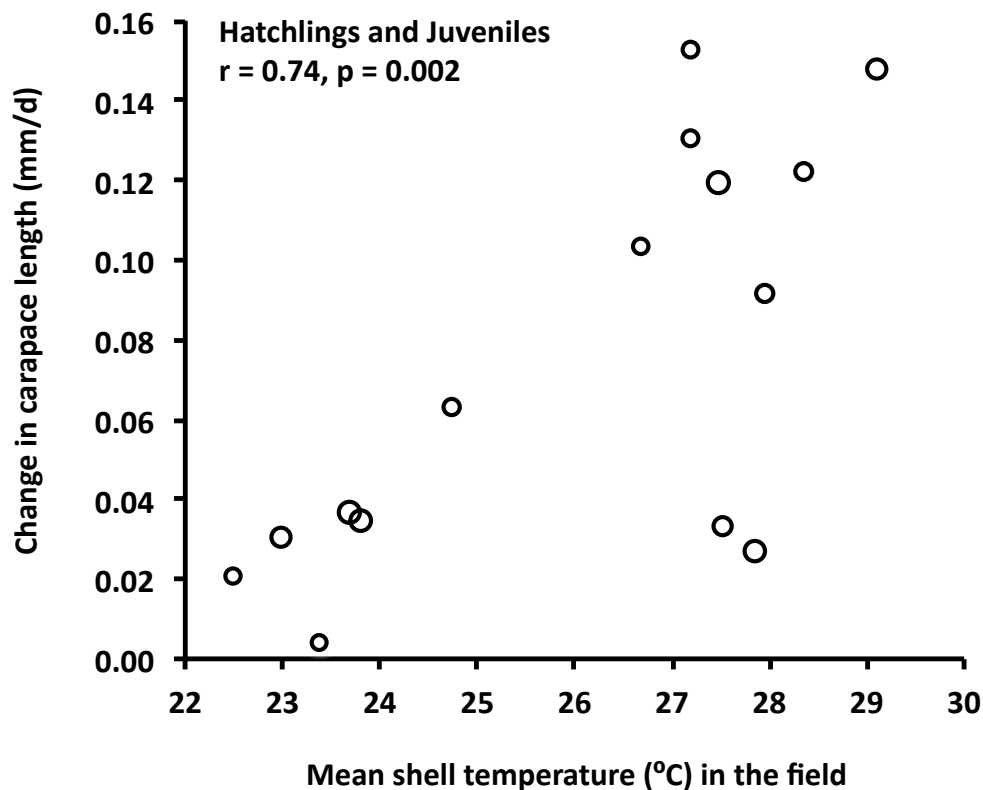


Figure 0-8. Relationship between growth rate (carapace length) and mean shell temperature for hatchling (0.5–1.0-year-old) and juvenile (1.0–4.0-year-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. Paired growth rates and shell temperatures were obtained by fitting free-ranging tortoises with iButton miniature temperature loggers in April–July and recapturing individuals 30–35 days later. Symbol size reflects initial carapace length, a covariate in the Pearson partial correlation analysis shown above.

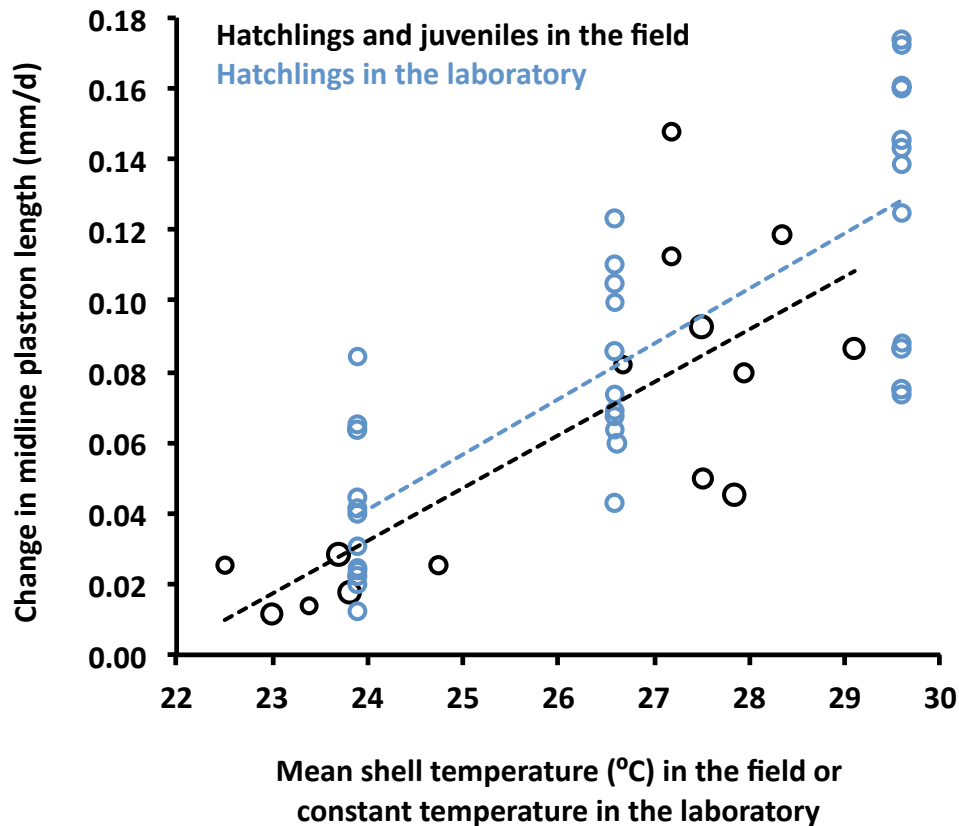


Figure 0-9. Relationship between growth rate (midline plastron length; MPL) and mean shell temperature for free-ranging hatchling (0.5–1.0-year-old) and juvenile (1.0–4.0-year-old) gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA (black). Relationship between growth rate (MPL) and constant temperature treatment in the laboratory for hatchling gopher tortoises under unlimited food regime (blue). Paired growth rates and shell temperatures were obtained by fitting free-ranging tortoises with iButton miniature temperature loggers in April–July and recapturing individuals 30–35 days later. Symbol size reflects initial carapace length for both field and laboratory animals. Fit lines are linear least square regressions.

CHAPTER 3: BEHAVIOR AND TEMPERATURE MODULATE A THERMOREGULATION-PREDATION RISK TRADE-OFF IN JUVENILE GOPHER TORTOISES

Introduction

Many ectotherms regulate body temperature behaviorally, which can enhance physiological processes such as digestion and growth, but thermoregulatory activities, including basking and shuttling movements between sun and shade, can expose individuals to increased predation risk (Lillywhite et al. 1973, Huey 1974, Huey and Slatkin 1976, Angilletta 2001). These and other costs may influence the extent to which reptiles thermoregulate and can impose constraints on physiological performance and life history traits (Huey 1974, Huey and Slatkin 1976, Vickers et al. 2011, Sears et al. 2016). For example, predator scent caused garden skinks (*Lampropholis guichenoti*) to spend less time in optimal foraging and thermoregulatory microhabitats and to grow more slowly (Downes 2001). Similarly, some snakes limit basking as juveniles, possibly in response to avian predators, a thermoregulatory strategy that may result in lower growth rates (Webb and Whiting 2005, Lelièvre et al. 2013). However, limited basking may not be an optimal, or even demographically feasible, strategy if juveniles grow very slowly and remain vulnerable to predators during other activities (e.g., sheltering in refugia or foraging).

Although basking in open habitats can increase exposure to predators, organismal traits such as cryptic coloration and specialized sensory capacities can reduce predation risk during thermoregulation (Robert and Thompson 2007, Vitousek et al. 2007, Smith et al. 2016). Ectotherms can also optimize trade-offs between costs and

benefits of thermoregulatory behavior that may vary with environmental conditions through careful microhabitat selection and other responses (Huey 1974, Huey and Slatkin 1976). Considerable data support the hypothesis that prey, including basking individuals, modify flight initiation distance (FID) to balance predation risk against flight costs (Ydenberg and Dill 1986, Cooper and Blumstein 2015). Costs and benefits can also influence subsequent refuge use. For example, after entering a refuge in response to a predator, some lizards hide longer when the refuge is warm or when external temperatures are low, presumably because physiological costs of refuge use are not as great (Martin and López 1999, Martin and Lopez 2001, Amo et al. 2003, Cooper and Wilson 2008, Martín and López 2010). Similarly, in warm environments, ectotherms may be able to maintain preferred body temperatures without having to bask and expose themselves to predators as much as when basking (Webb and Whiting 2005, Akins et al. 2014).

Most investigations of trade-offs to predator avoidance in reptiles focus on lizards (Downes 2001, Cooper and Blumstein 2015, Martín and López 2015). Comparatively, turtles are understudied in this regard (Cooper 2015), and even FIDs remain largely undocumented (but see Costa 2014, Polich and Barazowski 2016), especially for juvenile turtles. Studying how organismal traits and environmental conditions influence a trade-off between thermoregulation and predator avoidance in turtles provides an opportunity to assess the generality of relevant predator avoidance hypotheses (e.g., Ydenberg and Dill 1986, Lima and Dill 1990, Martín and López 2015) in a distinct lineage and potentially reveal novel evolutionary responses to a fundamental problem confronting many ectotherms (Cooper and Blumstein 2015). In

contrast to many studies of small lizards (Blouin-Demers and Nadeau 2005, Sears et al. 2016), few studies of turtles report precise thermoregulation, but this may reflect the scarcity of field investigations into the thermoregulatory behavior of small juvenile chelonians. Moreover, investigating temperature relations in turtles is important because as climate change progresses, turtles may encounter not only first-order effects on activity, development, and sex ratios (Janzen 1994, Hawkes et al. 2007, Van Houtan et al. 2015), but potentially second-order impacts on predator–prey interactions, which remain far less examined (Lovich et al. 2014, Cavallo et al. 2015).

The gopher tortoise (*Gopherus polyphemus*), a threatened burrowing herbivore of the southeastern USA (USFWS et al. 2013), has experienced dramatic changes to its thermal environment. Fire suppression and other human activities have led to grand-scale conversion of longleaf pine (*Pinus palustris*) savannas, highly biodiverse ecosystems that once dominated the southeast and are hypothesized to be primary ancestral habitats of the gopher tortoise, to more shaded, cooler environments (Guyer and Hermann 1997, Van Lear et al. 2005, Watson and Gough 2012). Small juvenile gopher tortoises spend almost all of their time in or near self-constructed burrows that provide individuals protection against temperature extremes and a variety of predators, including snakes, mammals, and birds (Wilson 1991, Wilson et al. 1994, Pike and Grosse 2006, Perez-Heydrich et al. 2012). Although the literature is mixed regarding the extent to which juvenile gopher tortoises bask outside the relative safety of their burrows, multiple studies indicate that juveniles regularly bask on the bare patch of soil located just in front of their burrows (referred to as the burrow “apron”) and experience an important trade-off between thermoregulation and

predator avoidance (Wilson 1991, Wilson et al. 1994, Johnston 1996, Pike and Grosse 2006, Harris et al. 2015). Some authors also suggest that this trade-off may be amplified in cool months if young must bask more to maintain elevated body temperatures (Wilson 1991, Wilson et al. 1994). However, information regarding how young tortoises balance thermoregulation and predator avoidance, and how this dynamic may be influenced by thermal environment, is unavailable.

Here, I examine potential behavior and temperature influences on a thermoregulation–predation risk trade-off in hatchling and juvenile gopher tortoises. At my study site in southwest Georgia (USA), juvenile tortoises bask extensively just in front of their burrows to maintain body temperatures within preferred ranges (Chapter 2). I considered tortoises to be at greater risk of predation while basking outside of burrows than when inside burrows (Wilson 1991, Wilson et al. 1994). I investigate: (i) basking tortoise responses to simulated predator approach; (ii) potential associations between temperature and hiding time following approach; and (iii) potential associations between temperature and surface activity following approach. I predicted: (i) basking tortoises would hide inside burrows when approached and exhibit substantial FIDs, even if they were unable to visually observe the approaching researcher; (ii) hiding time following disturbance would correlate positively with burrow temperature; and (iii) amount of surface activity during the hour following disturbance would correlate negatively with surface and burrow operative temperatures.

Materials and Methods

I studied tortoise behavior on Arcadia Plantation (30°45' N, 84°0' W), located in Thomas County in southwest Georgia, USA. The site (957 hectares) was dominated by mature (>80 years) longleaf pine forest and included the Wade Tract, an 80 hectare old-growth ecological preserve that has never been heavily cut and contains many old trees (>200 years; Platt et al. 1988). Ground vegetation on both the Wade Tract and surrounding areas of Arcadia Plantation was dominated by wiregrass (*Aristida stricta*), oaks (*Quercus* spp.), and a diverse assemblage of other native plants. Soils at the site were a mix of sand and clay. The study area was managed using frequent prescribed fire (≤ 2 -year return intervals).

Simulated predator approaches

During April–August 2013, I performed systematic approaches on 20 basking hatchling (<1 year) or juvenile (1–4 years; aged following Aresco and Guyer 1998) gopher tortoises. Carapace lengths and masses of approached individuals were approximately 54–106 mm and 39–243 g, respectively. Most approached tortoises were recently captured (but not within at least 19 days preceding approaches) and fitted with miniature radio transmitters with short, flexible trailing antennas and iButton (Maxim Integrated Products, Sunnyvale, CA, USA) temperature loggers for another study. For the smallest study animals, post-winter hatchlings, combined equipment and epoxy mass totaled ~4–5 g, or ~6%–10% of body mass ($n = 3$ individuals with a transmitter and iButton and 3 with only an iButton) and did not noticeably affect behavior or

movement, including time required to enter burrows following disturbance (always <2 seconds in this study) or righting ability following naturally occurring overturning events captured on video (Chapter 4). Hatchlings fitted with equipment and measured 1 month later ($n = 5$ individuals) increased in carapace length ($\bar{x} = 3.3$ mm, range = 0.6–4.9 mm), midline plastron length ($\bar{x} = 3.0$ mm, range = 0.8–4.7 mm), and in four of five instances, mass ($\bar{x} = 8.3$ g, range = -1.4–15.0 g).

Prior to the approach day, I set up two video cameras at the focal burrow: one to record tortoise activity and a second to determine from a distance when the focal tortoise was basking to properly time my approach. A time-lapse video camera (Plotwatcher Pro, Day 6 Outdoors, Inc., Columbus, GA, USA) continuously (1 frame per second) recorded tortoise activity during daylight hours at the focal burrow. The camera was mounted to a 1.2- m wooden pole, pushed ~0.2 m into the ground ~1 m from the burrow. Using a similar pole setup, I positioned a surveillance camera that facilitated remote viewing of tortoise activity from 108 m away via a coaxial cable connected to a liquid crystal display (LCD) monitor.

I performed all approaches on sunny days, between 1100 and 1500 hours, always wearing the same clothing type and color. Before each approach, I used the remote camera and LCD monitor to determine from 108 m away whether the focal tortoise was on its burrow apron. When the tortoise was observed on the apron, I initiated a steady walk (~1.5 m/s; speed determined by recording walking time in most approaches) toward the burrow, following the outstretched coaxial camera cable to ensure a straight path. After reaching the focal burrow, I briefly placed my hand in front of the video camera to facilitate later determination of how much time passed between when the

tortoise initiated a flight response and when I arrived at the burrow, information that was used in conjunction with approach speed to estimate FID (see below). I then immediately turned around and left the area, allowing the tortoise to reemerge undisturbed.

On several occasions, I mistook a shadow or other feature on the video feed for a tortoise basking on the burrow apron and approached the burrow when the individual was either belowground or away. In one instance, a tortoise left the burrow area just as I initiated my approach. These individuals were approached again on a subsequent day. Additionally, I excluded one approach and repeated it on another day because the burrow temperature logger malfunctioned.

Operative temperatures

Prior to approach days, I set out recording devices to measure burrow and apron operative temperatures. I used an iButton miniature temperature logger to record temperature 10 cm inside the burrows of approached tortoises at 10 minute intervals. This distance inside the burrow represents the warmest location available to tortoises within burrows during times of day when I performed approaches (T.A. Radzio, unpublished data). The iButton was affixed with epoxy to the end of a thin wooden spike such that the two objects created a nail-like shape. I then pushed the wooden spike into the sidewall of the burrow until the iButton laid securely flat against it.

I used simple operative temperature models to record operative temperatures on burrow aprons of approached tortoises. Models consisted of thin-walled aluminum jars with aluminum lids, measuring 2.6×5.0 cm (small models used on the aprons of 0.5- to

2.0-year-old turtles) or 4.3×8.2 cm (large models used on the aprons of 2.5- to 4.0-year-old turtles), and painted gray using Rust-Oleum Painter's Touch 2x Ultra Cover flat gray primer (model number 249088). An iButton temperature logger, epoxied inside the center of the hollow models atop a small plastic spacer, recorded apron operative temperatures at 10 minute intervals.

To assess the accuracy of operative temperature models, I compared small model temperatures to the internal temperatures of a dried neonate gopher tortoise carcass set out in an open field. I recorded carcass internal temperature by inserting a small thermistor, connected by wire to a temperature logger (HOBO 64K Pendant Waterproof Temperature/Alarm Data Logger; Onset Computer Corporation, Bourne, MA, USA), into the body cavity through a small hole in the skin above a hind limb that was sealed with silicone. I compared temperatures of a small model and the carcass at 5 minute intervals in an open field during midday hours on three partly cloudy days during March–September. Operative temperatures during testing times ranged between 20 and 55°C. The mean difference between temperatures recorded by metal models and the dry carcass was 1.8 ± 1.5 (1 SD) °C. Differences did not exceed 4.5°C, except briefly during very rapid temperature changes when they differed by as much as 8.2°C, which I largely attribute to differences in the heat capacity of the heavier metal model and the dry hatchling carcass.

Validation of operative temperature model accuracy is incomplete, particularly with regard to operative temperatures in rapidly changing environments, for older, larger-sized juvenile tortoises. However, large and small models recorded nearly identical temperatures. Predictably (Gates 1980), large models recorded higher temperatures in

full sun conditions, but only by $\sim 1\text{--}2$ °C. I conclude that in most cases models were satisfactory for my need, which was assessing if surface operative temperatures would allow basking tortoises to reach preferred body temperatures.

Statistical analyses

I estimated FID as the product of approach speed (1.5 m/s) and number of seconds between when an approached tortoise initiated flight response and when the approaching researcher arrived at the burrow (both documented by the video camera). I tested for relationships between FID and tortoise age, carapace length, and mass using Pearson correlations. To visually represent the relationship between FID and tortoise age, I used a linear least square regression (LLSR). I visually inspected data to assess linearity and homoscedasticity and performed logarithmic transformations where appropriate.

I quantified two measures of hiding time: “appearance” time (when a tortoise’s head first appeared in the burrow entrance) and “reemergence” time (when the tortoise’s head fully emerged out of the burrow). Young gopher tortoises routinely bask in burrow entrances with only their heads and a portion of their body above ground (T.A. Radzio and M.P. O’Connor, unpublished data). In most instances, appearance and reemergence times were very similar and preceded further movements out of the burrow seconds later. However, after hiding for 3.7 minutes in response to my approach, one tortoise extended its nose out of the burrow entrance for only 1 second, but was not visible again until 12.4 minutes later when it reemerged with its head fully out of the burrow. I tested for relationships between burrow temperature at

10 cm, the warmest temperature available to tortoises within burrows during approach times (T.A. Radzio, unpublished data), and appearance time and reemergence time by performing Pearson partial correlations (PPC; Package “ppcor” in R, Kim 2015) with age as a covariate. To satisfy linearity assumptions, I performed these analyses on log-transformed hiding times. I first conducted the above analyses using data from all approaches ($n = 20$). However, disturbed tortoises may be less inclined to reemerge when surface operative temperatures are low. Therefore, to better isolate potential burrow temperature associations with hiding time, I repeated the analyses using only approaches where apron operative temperatures were $>33^{\circ}\text{C}$ ($n = 17$), the approximate preferred body temperature of fed hatchling gopher tortoises (Johnston 1996; Chapter 2). I visually represent simple relationships between burrow temperature and log hiding times without accounting for possible covariate effects by performing LLSRs.

I tested for relationships between surface activity during the hour following researcher approach and two variables, mean burrow temperature at 10 cm, and mean apron temperature during the hour following approach, by performing PPCs with tortoise age as a covariate. I visually represented simple relationships between burrow temperature and surface activity during the hour following researcher approach without accounting for possible covariate effects by performing LLSRs. I conducted all analyses in RStudio version 1.0.136 (RStudio Team 2016). Statistical tests were two-tailed, with $\alpha = 0.05$.

Results

Response to researcher approach

Tortoises on burrow aprons or in burrow entrances ($n = 20$) always responded to my approach by entering burrows. Mean FID was 45 ± 22 (1 SD) m (range = 13–101 m) and correlated positively with age (PC, $r = 0.84$, $df = 18$, $p < 0.00001$, Figure 1), carapace length (PC, $r = 0.81$, $df = 17$, $p = 0.0001$), and mass (PC, $r = 0.82$, $df = 17$, $p = 0.0001$). Individuals did not make movements prior to initiating flight that would suggest substantial differences between FIDs and alert distances.

Upon initiating flight response, tortoises quickly entered burrows without first changing body orientation. In other words, if a tortoise was facing away from the burrow entrance, it quickly backed into the burrow, rather than first turning around and then entering. On average, tortoises filmed at 1 frame/s ($n = 19$) completely entered burrows within at least 1.7 seconds of initiating flight (range: 1–3 seconds). One tortoise, inadvertently filmed at 0.2 frames/second, completely entered the burrow within at least 5 s of initiating flight (the fastest measurable speed given the reduced frame rate). On average, tortoises entered burrows in a similar rapid manner only 0.7 times (median = 1, range = 0–2, $n = 20$ tortoises) in the 60 minutes of burrow entrance or apron activity preceding simulated predator approach. Cameras did not document any potential predators when tortoises made these rapid movements into burrows. These movements were not in response to my presence because I avoided focal tortoises prior to approaches.

Hiding time

On average, tortoises appeared in burrow entrances 18.3 ± 11.7 minutes (3.4–50.0 min; $n = 20$) following disturbance. Log appearance time was not partially correlated with burrow temperature at time of approach (PPC with age as a covariate, $r = 0.42$, $df = 17$, $p = 0.074$, Figure 2a), but log reemergence time was (PPC with age as a covariate, $r = 0.52$, $df = 17$, $p = 0.022$, Figure 2b). Similarly, when only approaches with apron operative temperatures exceeding 33°C were included in the analyses ($n = 17$), log appearance time was not partially correlated with burrow temperature (PPC with age as a covariate, $r = 0.49$, $df = 14$, $p = 0.052$, Figure 2c), but log reemergence time was (PPC with age as a covariate, $r = 0.64$, $df = 14$, $p = 0.007$, Figure 2d). Understandably, burrow temperature also correlated with approach time (PC, $r = 0.76$, $df = 18$, $p < 0.001$), day of year (PC, $r = 0.83$, $df = 18$, $p < 0.00001$), and apron temperature (PC, $r = 0.82$, $df = 18$, $p < 0.00001$).

Surface activity during the hour following approach

Surface activity during the hour following approach was almost entirely (525.8 of 547.4 minutes) restricted to burrow entrances and aprons and consisted primarily of basking and movements in and out of burrows. In fact, only 1 of 20 tortoises engaged in surface activity away from its burrow entrance or apron. Surface activity during the hour following approach exhibited a negative partial correlation with mean burrow temperature (PPC with age as a covariate, $r = -0.55$, $df = 17$, $p = 0.015$, Figure 3), but not mean apron operative temperature during the hour following approach (PPC with age as a covariate, $r = -0.43$, $df = 17$, $p = 0.069$). Mean burrow temperature during the

hour following approach also correlated with day of year (PC, $r = 0.84$, $df = 18$, $p < 0.00001$), approach time (PC, $r = 0.74$, $df = 18$, $p < 0.0001$), and mean apron operative temperature (PC, $r = 0.74$, $df = 18$, $p < 0.0001$).

Discussion

Combining simulated predator approaches with remote video observations revealed exceptionally long FIDs ($\bar{x} = 45$ m) for young gopher tortoises, much greater than typically reported for other small reptiles (Cooper et al. 2014, Costa 2014; but see Legler and Vogt 2013, Polich and Barazowski 2016; Figure 1). Due to dense herbaceous vegetation and long FIDs, I never visually observed a tortoise before it hid inside its burrow. Similarly, I believe that in all or most cases it was not possible for tortoises to visually observe me, especially in two instances, where tortoises were oriented toward burrows with their heads completely belowground when approached (FIDs = 49 and 52 minutes). Thus, I hypothesize that young gopher tortoises use vibrational cues (aerial or ground) to detect predators early and reduce predation risk associated with basking. Long FIDs may be common in turtles (Legler and Vogt 2013, Polich and Barazowski 2016), but very few data are available for this group. Optimal escape theory predicts that prey will modify FID to balance potential costs and benefits of fleeing (Ydenberg and Dill 1986, Cooper and Blumstein 2015). A unique factor potentially contributing to the long FIDs and rapid flight movements of young gopher tortoises may be that individuals are unable to always reliably assess if vibrational cues correspond to a “loud” predator that is far away or a “quiet” one that is nearby and often hide immediately upon detecting any irregular or signature vibration.

Detecting potential predators via non-visual cues seems particularly useful in longleaf pine ecosystems where a dense herbaceous layer limits line-of-sight for small ground-dwelling animals. However, tortoises may be less perceptive to vibrational cues when walking (e.g., manicuring burrow aprons, foraging) or in noisy environments (e.g., high winds, near highways, or foraging conspecifics). Tortoises may also waste energy and miss thermoregulatory opportunities by responding incorrectly to vibrations that are not associated with a potential predator. Video-recorded hatchlings routinely flee into burrows in response to non-threatening animals, such as fox squirrels (*Sciurus niger*) and adult tortoises (T.A. Radzio, unpublished data). In this study, basking tortoises made on average 0.7 rapid, presumably antipredator, movements into burrows during the hour of surface activity preceding simulated predator approach, but in no instance ($n = 13$) was an actual predator observed visiting burrows. Despite potential energetic and thermoregulatory costs, young tortoises appear to err on the safe side and hide inside burrows in response to false-positive threat cues, perhaps because failing to respond to a real threat can be lethal.

Vigilance and apparent use of vibrations to detect potential predators suggest an ecological function for the greatly exaggerated inner ear morphology of gopher tortoises. Bramble (1982) describes the “enormous” otolith (ear bones) of gopher tortoises to be larger than those of any living tetrapod, and Bramble and Hutchison (2014) state that they are the species’ most striking feature, more so than any specializations for digging burrows (e.g., fused carpals). Among other potential functions, Bramble (1982) hypothesized that the massive otoliths and other specialized inner ear structures of gopher tortoises function as hypersensitive seismic sensors that

provide individuals inside burrows information about predator activity at the surface that can be used to decide when to safely emerge. I extend this hypothesis to include that they may reduce costs associated with extensive thermoregulatory activity by increasing the ability of basking individuals to detect predators and hide inside burrows before being attacked. Notably, the gopher tortoise's closest living relative, *G. flavomarginatus*, also exhibits very large otoliths and very long FIDs in response to humans (Bramble 1982, Legler and Vogt 2013, Bramble and Hutchison 2014).

Flight initiation distance increased with tortoise age and size, despite likely declines in predation risk as individuals become larger (Wilson 1991, Perez-Heydrich et al. 2012, Tuberville et al. 2014). In lizards, similar positive correlations between FID and body size, occurring within and among species, may reflect a greater reliance on crypsis for predator avoidance in smaller, slower-moving individuals (Cooper 2011, Cooper et al. 2014, Cooper 2015). Although there are other potential explanations, such as, perhaps, greater flight costs in smaller individuals, I am intrigued by the possibility that greater sensory constraints on the ability of younger tortoises to detect vibrations could underlie the ontogenetic pattern I observed. Hatchlings and small juveniles have soft shells, composed primarily of keratin, that do not fully ossify until tortoises are well beyond the sizes of the study animals (Wilson 1991). Perhaps as young gopher tortoises become larger, heavier, and more ossified, their ability to conduct and sense vibrational cues increases.

Consistent with a hypothesized thermoregulation–predator avoidance trade-off (Wilson 1991, Wilson et al. 1994, Harris et al. 2015), tortoises generally reappeared from burrows soon after hiding in response to simulated predator approach ($\bar{x} = 18.3$

minutes, Figure 2). Field observations at this site and a laboratory growth experiment indicate that hatchlings and juveniles usually must emerge from burrows and bask to achieve body temperatures at which growth rates are highest (Chapter 2). By limiting time in burrows following disturbance, tortoises can maintain high body temperatures and reduce physiological costs of predator avoidance.

This investigation is the first to examine whether turtles adjust hiding times in response to temperature. As documented in various lizard species (Martin and López 1999, Martin and Lopez 2001, Amo et al. 2003, Cooper and Wilson 2008, Martín and López 2010), thermal costs appear to constrain hiding time in hatchling and juvenile tortoises. Reemergence times were shorter when burrows were cooler, presumably because physiological costs were greater. I suspect that this phenomenon is more widespread in turtles, including aquatic species that terminate aerial basking in response to disturbance (Moore and Seigel 2006, Selman et al. 2013, Polich and Barazowski 2016). Adjusting hiding time in response to refuge temperature also suggests a precise thermoregulatory strategy. Indeed, at my study site hatchlings and juveniles exploit most thermal opportunities available at burrows and burrow aprons to maintain body temperatures within ranges that promote rapid growth (Chapter 2).

To my knowledge, this is also the first study to examine relationships between temperature and time spent outside refugia following real or simulated predator disturbance. This parameter, though requiring extended observation to measure, can be important because predation risk may remain elevated if a predator stays in the area, but prey do not always accurately assess predation risk upon reemergence. Surface activity during the hour following disturbance correlated

negatively with burrow temperature. Basking accounted for most surface activity, providing preliminary support for the hypothesis that thermal environment determines the minimum amount of time individuals must spend basking to maintain preferred body temperatures (Wilson 1991, Wilson et al. 1994), and thus, time outside burrows following disturbance. I conclude that burrow temperature and, perhaps to some extent, apron temperature influenced hiding time and surface activity following disturbance, and feel that time of day and day of year were simply correlated with burrow thermal environment.

Despite antipredator responses and extensive basking, hatchling and small juvenile gopher tortoises exhibit low survivorship, and individuals from forested sites at similar latitudes typically require approximately 15–20+ years to reach maturity (Landers et al. 1982, Wilson 1991, Aresco and Guyer 1999a, Perez-Heydrich et al. 2012), putting a premium on juvenile growth and survivorship. The ability to detect potential predators using non-visual cues likely reduces predation risk associated with thermoregulatory basking in hatchling and juvenile gopher tortoises. I speculate that hypertrophied otoliths and specialized inner ear morphology (Bramble 1982, Bramble and Hutchison 2014) may represent key sensory features allowing young gopher tortoises to exploit thermal opportunities required for rapid growth while also maintaining predator-induced mortality at demographically feasible levels. My data also indicate that in warmer environments young tortoises hide longer when disturbed and spend less time outside the relative safety of their burrows following disturbance. In the setting of temporal and thermal constraints on the energetics of ectothermic herbivores (Troyer 1987, Zimmerman and Tracy 1989, van Marken Lichtenbelt 1992,

Tracy et al. 2005), this trade-off between predator avoidance and basking highlights the importance of endangered longleaf pine ecosystems, where much solar radiation reaches and warms the ground, to this threatened species (Guyer and Hermann 1997, Van Lear et al. 2005, Watson and Gough 2012).

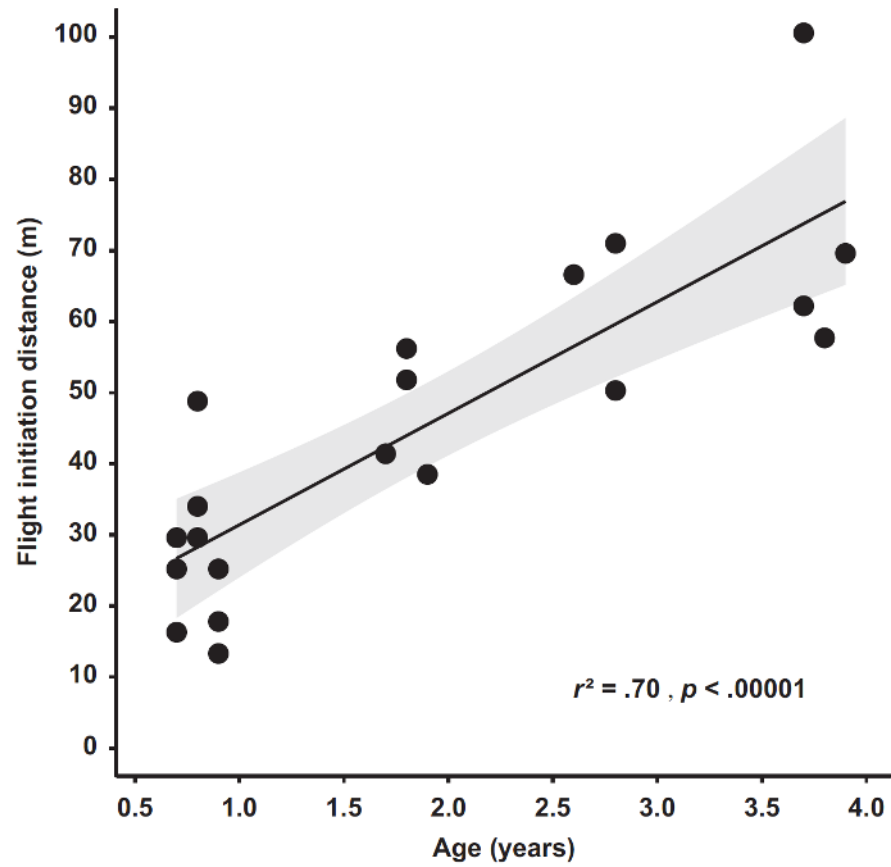


Figure 0-1. Relationship between gopher tortoise age and flight initiation distance in response to researcher approach. Solid line depicts linear least square regression, and shading represents 95% confidence interval.

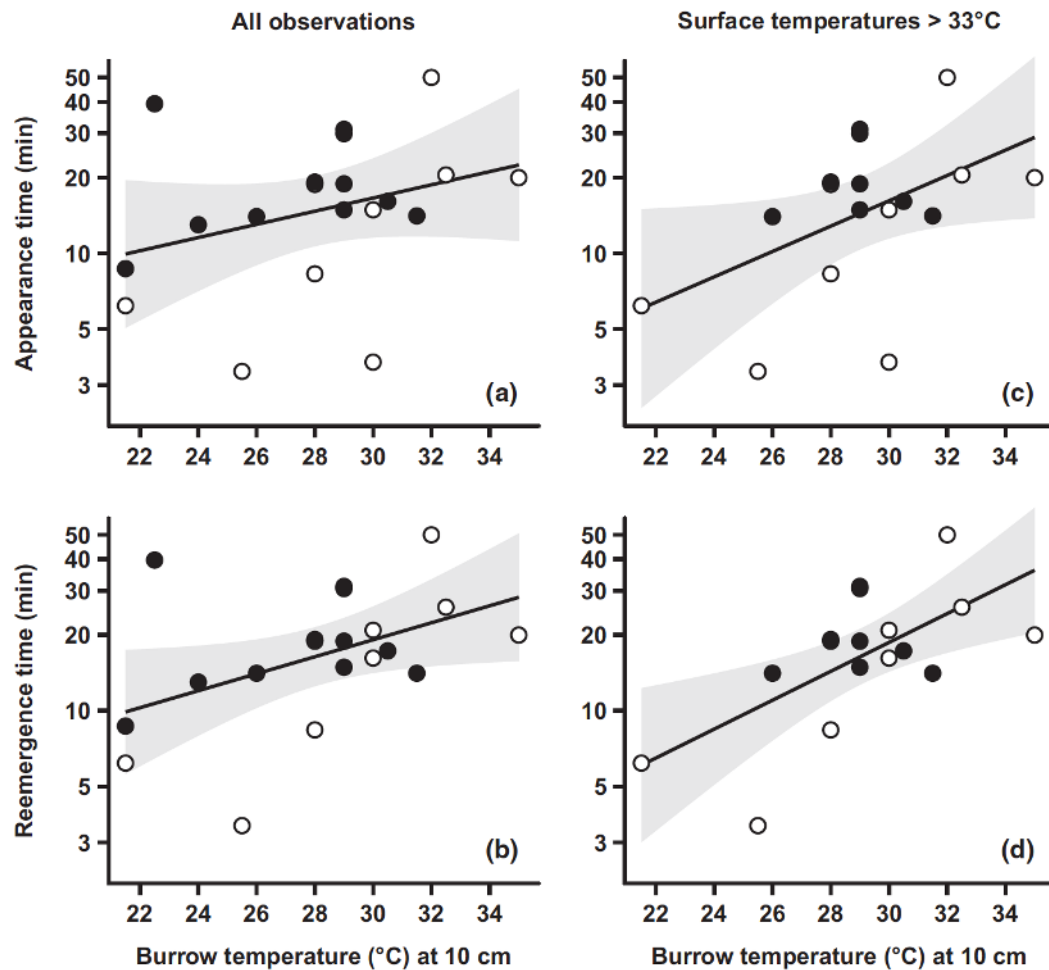


Figure 0-2. Relationships between: (a) burrow temperature (at 10 cm) and gopher tortoise appearance time for all observations (linear least square regression [LLSR], $r^2 = .10$, $p = .173$); (b) burrow temperature and gopher tortoise reemergence time for all observations (LLSR, $r^2 = .21$, $p = .044$); (c) burrow temperature and appearance time for observations with burrow apron operative temperatures $>33^\circ\text{C}$ (LLSR, $r^2 = .25$, $p = .042$); (d) burrow temperature and reemergence time with burrow apron operative temperatures $>33^\circ\text{C}$ (LLSR, $r^2 = .42$, $p = .005$). Statistics are performed on log-transformed hiding times (note log scales on y-axis). Open circles represent <1 -year-old tortoises, and filled circles represent 1- to 4-year-old individuals. Solid lines are linear least square regression lines, and shading represents 95% confidence intervals. See text for alternative Pearson partial correlation analyses that control for potential age effects.

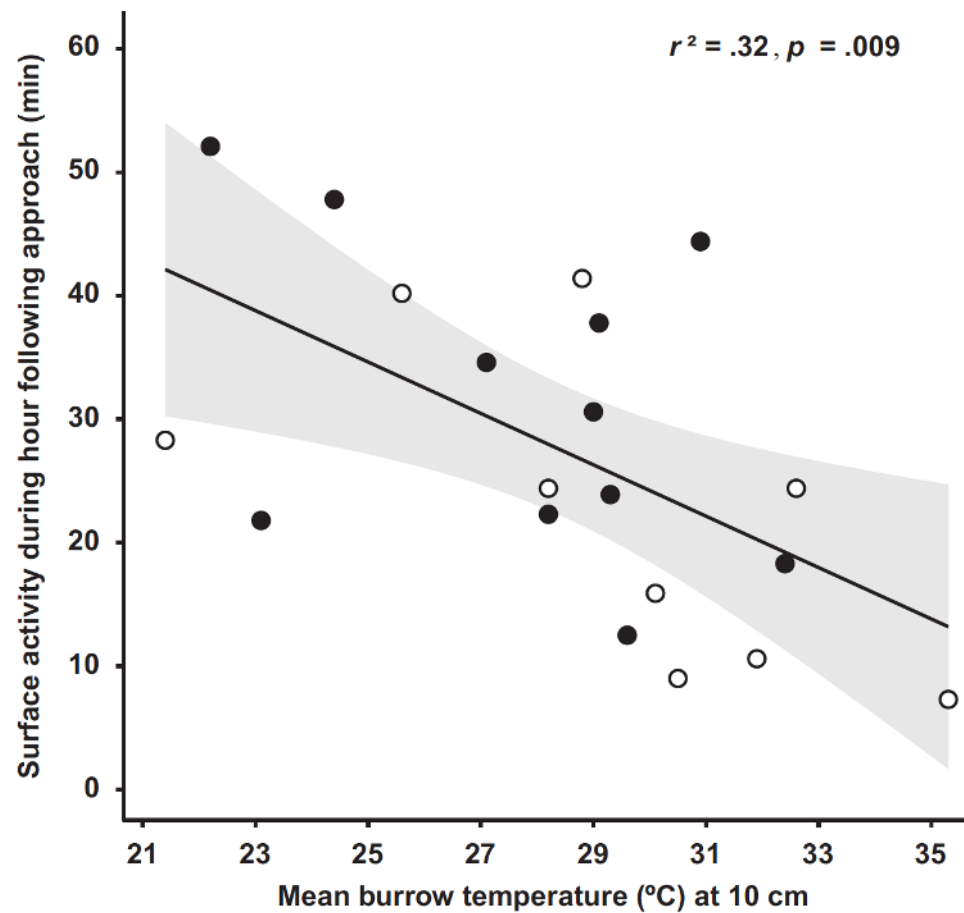


Figure 0-3. Relationship between mean burrow temperature at 10 cm and immature gopher tortoise surface activity during the hour following researcher approach. Open circles represent <1-year-old tortoises, and filled circles represent 1- to 4-year-old individuals. Solid line depicts linear least square regression, and shading represents 95% confidence interval. Surface activity was confined almost entirely (96.1%) to burrow entrances and aprons. See text for an alternative Pearson partial correlation analysis that controls for potential age effects.

CHAPTER 4: ACTIVITY TEMPERATURES, OVERTURNING IN NATURE, AND CONSTRAINTS ON THE RIGHTING RESPONSES OF HATCHLING GOPHER TORTOISES

Introduction

Turtles can overturn when negotiating uneven terrain and during interactions with conspecifics or predators (Burger 1976, Hosier et al. 1981, Steyermark and Spotila 2001, Limpus et al. 2003). Their shells and short limbs can hinder righting (Bonnet 2001, Zuffi and Plaitano 2007, Golubović et al. 2013b, Golubović 2015), and overturned individuals may experience dangers such as increased vulnerability to predators, desiccation, and overheating (Bonnet 2001, Limpus et al. 2003, Corti and Zuffi 2003, Holbrook et al. 2015, Golubović 2015). Righting performance can vary among populations, between sexes, and with ontogeny (Golubović et al. 2013b, Golubović 2015) and may play a role in shaping turtle behavior by influencing risk associated with activities that can lead to overturning. Righting quickly may be particularly important for small turtles active in hot terrestrial environments because their low thermal inertia can lead to rapid overheating (Burger 1976).

How quickly an overturned turtle rights depends on how long it waits to initiate the righting attempt (latency time) and how much time it subsequently needs to perform the mechanical righting maneuver (active righting time). Despite potential dangers associated with remaining overturned, turtles often exhibit long latency times in experiments (e.g., Steyermark and Spotila 2001, Polo-Cavia et al. 2012, Davy et al. 2014, Mitchell et al. 2016), perhaps because manually overturned individuals often prioritize antipredator behaviors, such as becoming still or withdrawing into shells, over

immediately attempting to right (Steyermark and Spotila 2001, Golubović 2015). In some species, the active righting maneuver is essentially automatic, but in others it can require considerable time and effort (Steyermark and Spotila 2001, Zuffi and Corti 2003, Davy et al. 2014) and can be influenced by a number of factors, including temperature (Freedberg et al. 2004, Elnitsky and Claussen 2006, Ben-Ezra et al. 2008, Polo-Cavia et al. 2012). Anecdotal accounts suggest certain substrates, such as loose sand, hinder righting in some species (Stancher et al. 2006), though I am unaware of studies investigating substrate effects on righting.

The gopher tortoise (*Gopherus polyphemus*) is a burrowing herbivore of the southeastern United States that inhabits semi-open canopy environments where substantial direct solar radiation reaches the ground (Douglass and Layne 1978, Diemer 1986). Surface operative temperatures routinely exceed lethal levels in tortoise habitats, but individuals avoid overheating by sheltering in burrows, long structures that also provide protection against predators (Douglass and Layne 1978, Johnston 1996). Hatchlings and juveniles bask extensively on the bare patch of soil located directly in front of burrows (referred to as the “burrow apron”) and typically feed away from burrows during midday hours, a foraging strategy that may reduce interactions with predators (Johnston 1996; Chapter 2). However, this can place individuals at risk of overheating if they overturn in direct sunlight and are unable to right quickly. Similar to adult tortoises, hatchling gopher tortoises also engage in aggressive combat with other hatchlings where they ram and sometimes overturn one another, ostensibly for access to burrows (Radzio et al. 2016; Chapter 5). Thus, righting response may play an important

role in multiple facets of hatchling gopher tortoise ecology, including influencing cost associated with activity in hot environments.

I investigated hatchling gopher tortoise shell and environmental temperatures during activity away from burrows, causes of overturning, righting responses in the field, and estimated how long individuals could remain overturned before reaching critical thermal maximum (CTM). I also assessed temperature and substrate effects on hatchling righting responses in the laboratory. I predicted hatchlings would exhibit shorter latency and active righting times at higher temperatures and would require longer to right on “loose sand” than on “structured sand,” loose sand augmented with a wire grid to provide additional grip for the active righting maneuver.

Materials and Methods

Study site

I made observations of hatchling gopher tortoises on Arcadia Plantation, a 957-ha private property located in southwest Georgia, USA. The site consisted of mature and old-growth longleaf pine (*Pinus palustris*) forest, managed using frequent prescribed burns (Platt et al. 1988). Ground cover was dominated by wiregrass (*Aristida stricta*), oaks (*Quercus* spp.), and a diverse assemblage of other native plants. Soils were a complex mosaic of sand and clay (NRCS Web Soil Survey 2016).

Temperatures during surface activity away from burrow aprons

In July 2012 and April–September 2013, I captured hatchlings using bucket traps at burrow entrances or by hand away from burrows. I define hatchlings as 0.5–1.0-year-

old-tortoises. However, observations subsequently made in September–October 2013 (n = 3 individuals) likely included one or more individuals that had very recently started their second year of life. I epoxied an iButton miniature temperature data logger (Maxim Integrated Products, Sunnyvale, California, USA), programmed to record temperature at 1.5-min (in 2012) or 5-min intervals (in 2013), to the rear vertebral scute of individuals. I also epoxied a miniature radio transmitter to either the right or left posterior costal scute. To avoid adversely affecting shell growth, I did not attach equipment to, or apply epoxy across, multiple scutes. Prior to attachment in 2013, I coated equipment with a thin layer of brown rubber (Plasti Dip International, Blaine, Minnesota, USA) to provide waterproofing and camouflage. I released individuals at their burrows within 24 hours of capture.

I identified periods of hatchling surface activity away from burrow aprons using time-lapse video cameras (Plotwatcher Pro, Day 6 Outdoors, Columbus, Georgia, USA), set to record activity at burrow entrances, burrow aprons, and a small area around burrows. Cameras were mounted atop wooden poles and recorded continuously (1 frame per second) throughout daylight hours. Using this approach, I obtained 578 shell temperature observations during 92 bouts of surface activity away from burrow aprons, initiated outside rain events, by 10 hatchlings (1 in 2012, 9 in 2013). I focused on surface activity away from burrow aprons outside rain events because I was primarily interested in determining whether overturning exposes individuals to overheating risk. During rainfall, young tortoises sometimes briefly leave burrows to drink water that temporarily accumulates on the ground (Chapter 2; Pike and Grosse 2006). Hatchling surface activity away from burrow aprons outside rain events likely consists primarily of foraging

(Chapter 2; Pike 2006). In 2013, I used metal models (described and validated in Radzio and O'Connor 2017; Chapter 3) to record operative temperatures at 10-minute intervals on burrow aprons of video recorded hatchlings and in a large open field at the study site.

Overturning in the field

During August 2011– June 2016, I intermittently recorded hatchling activities at burrows using time-lapse video cameras. This included both individuals fitted with iButton miniature temperature loggers (described above) and other hatchlings. I reviewed all videos and summarized all overturning events. Overturning observations (n = 15) were primarily of wild hatchlings (n = 9, one observed to overturn 3 times), but also included hatchlings (n = 4) raised for the first 11 months of their lives in the laboratory and subsequently released (Chapter 6). Ages of overturned hatchlings ranged between 0 to 11 months. Most (10 of 15) overturning events involved hatchlings fitted with an iButton and/or radio transmitter. Among overturned individuals, combined equipment and epoxy mass did not exceed 7% of body mass.

Laboratory animals

In September and October 2013, I collected 60 hatchling gopher tortoises as they emerged from nests at the study site. Additional descriptions of hatchling collection are provided in Radzio et al. (2017; Appendix 2). I maintained hatchlings in 53.0 × 8.5 × 3.7-cm (L:W:H) rectangular, black plastic enclosures. Most of each enclosure (43-cm length) was covered by a wooden board, providing individuals a long, dark and relatively cool burrow. A small portion of the enclosure (10-cm length) was only covered by a metal

screen, where hatchlings could bask under a suspended heat lamp. I provided hatchlings a diet consisting primarily of organic chicory (*Cichorium spp.*) and Zoo Med Natural Grassland Tortoise Food (Zoo Med Laboratories, Inc., San Luis Obispo, California). I soaked individuals in a shallow pan containing water, usually for at least 15 min, at least once every four days, but generally more frequently. I occasionally dusted food with Rep-Cal calcium and Herptivite supplements (Rep-Cal Research Laboratories, Los Gatos, California, USA.).

Light and temperature regimes differed minimally between when hatchlings were initially maintained in a field laboratory at Tall Timbers Research Station (early September–early November 2013) and during the following 3 months preceding the experiment when hatchlings were maintained in the laboratory at Drexel University (early November 2013–early February 2014). During the 3-month period directly preceding the experiment, hatchlings were kept on a 13:11 light:dark cycle with heat lamps over basking areas turned on from 1000–1700 hours to facilitate behavioral thermoregulation, in which hatchlings appeared to engage extensively. Five days per week, 150-W neodymium basking bulbs provided operative temperatures of $\geq 40^{\circ}\text{C}$ at the basking end of enclosures. Two days per week, 160-W Powersun UV mercury vapor bulbs (Zoo Med Laboratories, Inc., San Luis Obispo, California) provided lower operative temperatures and UVB radiation at the basking areas. When basking lamps were off, room air temperature remained between 22–26 $^{\circ}\text{C}$. Room air temperature rose when basking bulbs were on, and depending on location within the laboratory, temperatures at the cool end of burrows ranged between 24–30 $^{\circ}\text{C}$.

Laboratory righting experiment

I performed laboratory righting trials in 30 × 12 × 12-cm (L:W:H) plastic arenas located inside a large, well-lit, temperature-controlled incubator. I quantified righting responses at 5 temperatures: 20, 24, 28, 32, and 36 °C, and on two substrates, “loose” and “structured” sand. Both arenas were filled approximately one-third with dry play sand (Premium Play Sand #1113, The Quikrete Companies, Atlanta, GA). The structured sand treatment arena only differed from the loose sand treatment arena in that a 5-mm square wire mesh was buried even with, to slightly below (< 3 mm), the sand surface. The structured sand treatment was designed to simulate sand that provided substantial grip for righting (*sensu* Stancher et al. 2006), such as packed sand or sand containing fine roots.

I randomly selected 30 hatchlings for loose sand trials and 30 for structured sand trials and randomly subdivided individuals within each treatment into 5 groups of 6. After testing individuals from the first two groups on both substrates (12 hatchlings on loose sand and 12 hatchlings on structured sand) at each of 5 temperatures, it became apparent that hatchlings were essentially unable to right on loose sand (hatchlings failed to right on loose sand in 58/60 trials), and I discontinued loose sand trials. For hatchlings tested on loose or structured sand, mean carapace length was 59.0 ± 2.2 mm (1 SD; range = 54.2–64.0 mm), and mean mass was 52.0 ± 5.4 g (range = 42.7–62.1 g). Loose sand hatchlings (n = 12) represented 8 clutches (or mothers), and structured sand hatchlings (n = 30) represented 14 clutches (or mothers). Clutches represented distinct mothers because female gopher tortoises only nest up to once annually in southwest Georgia (Landers et al. 1980).

I tested individuals once per day, thus requiring at least five days to test a group of turtles at each temperature. Righting trials for the first and second groups of loose sand and structured sand hatchlings proceeded in the following order: 20, 24, 28, 32, and 36 °C; 24, 28, 32, 36, and 20 °C. Righting trials for the third through fifth group of structured sand trials ordered: 28, 32, 36, 20, and 24 °C; 32, 36, 20, 24, and 28 °C; and 36, 20, 24, 28, and 32 °C.

At least 12 h before each trial, I set the incubator to experimental temperature and placed a loose sand and a structured sand arena inside. For 32 and 36 °C trials, I used a space heater to warm the room to ≥ 28 and ≥ 32 °C, respectively, to minimize temperature changes when I briefly opened the incubator door between experimental trials. From 0810–0950 hours EST on trial days, I placed 6–12 scheduled hatchlings into holding containers at randomly assigned locations throughout the incubator. Each holding container also held an iButton miniature temperature data logger (Maxim Integrated Products, Sunnyvale, California, USA), programmed to record temperature at 10-minute intervals. Tortoises equilibrated to incubator temperature for 3.8–6.3 hours before righting trials were performed between 1220–1530 hours.

For each righting trial, I briefly opened the incubator door, picked up the scheduled hatchling, checked that it was awake (eyes open), and, if awake, placed the tortoise on its carapace inside its test arena (loose or structured sand). If the scheduled hatchling's eyes were closed (a rare occurrence), I waited at least 10 minutes before reattempting the trial. A time-lapse video camera (Plotwatcher Pro, Day 6 Outdoors, Columbus, Georgia, USA) mounted above test arenas recorded righting responses. After 10 min, I briefly opened the incubator, removed the hatchling, and placed the next

scheduled individual on its carapace in the appropriate test arena before closing the incubator door. Thermocouple measurements inside the incubator and iButton recordings in 2 experimental arenas and 12 holding containers indicated that briefly opening the incubator door had negligible effects on temperature inside the incubator. After the experiment, I determined using a certified mercury glass thermometer that iButtons were accurate to within ± 0.6 °C at 0, 30, and 45 °C.

Data analysis

I summarized hatchling shell temperatures, operative temperatures on burrow aprons, and operative temperatures in an open field during periods of surface activity away from burrow aprons, initiated outside rain events. Prior to performing calculations, I standardized shell temperature sampling rate across years (recorded at 1.5-minute intervals in 2012 and 5-minute intervals in 2013) by downsampling 2012 observations to approximately 5-minute intervals.

For each time-paired shell and environmental temperature (apron or open field) recorded during activity away from burrows, I estimated how long it would take a hatchling to reach CTM (43.9 °C, Hutchison et al. 1966) if it overturned and failed to right. Time to CTM was estimated by:

$$T = \tau \ln ([T_e - T_{\text{shell}}] / [T_e - \text{CTM}]),$$

where T equals time (min) to CMT, τ equals a mass-specific thermal time constant ($\ln[\tau] = 0.72 + 0.36 \ln[\text{mass}]$; Grigg et al. 1979), T_e equals operative temperature, and T_{shell}

equals shell temperature, assumed to closely approximate internal body temperature of hatchlings. This analysis likely represents worst case scenarios for two reasons. First, overturned hatchling gopher tortoises possess emergency physiological responses such as frothing and bladder voiding that can provide some short-term buffering against rising body temperatures (Douglass and Layne 1978, Johnston 1996). Second, the mass-specific thermal time constant I used was developed from lizards (Grigg et al. 1979). I am unaware of estimates of thermal time constants for small turtles. Available data indicate that within size ranges considerably larger than those of my study animals, turtles have larger thermal time constants than do lizards, perhaps related to conductivity properties of their thick shells (Grigg et al. 1979). However, shells of hatchling gopher tortoises are very thin, composed primarily of keratin, and largely not ossified (Wilson 1991).

I summarized causes of overturning and righting responses in the field. I calculated time to CTM for the one field overturning instance where operative temperature was measured and exceeded CTM. I defined latency time as time from when a hatchling overturned to when it initiated righting movements with its head or legs. I defined active righting time in the field as time from when a hatchling initiated a righting attempt until it righted. Video cameras in the field recorded at variable frame rates, ranging between 0.17–1.0 frames per second. Therefore, I calculated minimum (Field Min) and maximum (Field Max) latency time estimates for field observations. I only calculated maximum active righting times because individuals generally required longer to complete the active righting maneuver, rendering variation in video frame rate less important.

I reviewed laboratory righting trial videos to quantify latency and active righting times. I defined latency time in the laboratory experiment in the same manner as for field observations. In one trial, a hatchling did not attempt to right and was assigned a latency time of 600 seconds. I defined active righting time as time from when a hatchling initiated a righting attempt until it righted, or if it did not right, until the end of the 10-minute trial.

I investigated substrate effects on righting success in the laboratory using a Mann-Whitney U test. For this analysis, I used individual turtles as the sampling units and calculated a mean righting score for each turtle as the proportion of 10-minute trials over 5 test temperatures in which it righted. I used Friedman non-parametric tests for repeated measures (individual tortoises) to investigate temperature effects on latency and active righting times in the laboratory. I performed pairwise post-hoc comparisons using Wilcoxon signed rank tests with Bonferroni adjustments. For the analysis of temperature effects on latency time, I pooled observations of tortoises overturned on loose and structured sand. For the analysis of temperature effects on active righting time, I only used righting trials performed on structured sand because tortoises were essentially unable to right on loose sand. I used Mann-Whitney U tests to determine if latency and active righting times following naturally occurring overturning events in the field differed from responses in the laboratory. For comparisons of active righting time in the field and the laboratory, I only used field observations where individuals overturned fully onto their carapace. Statistical tests were two-tailed and were evaluated at $\alpha = 0.05$. I performed all statistical analyses in SPSS version 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 24.0. Armonk, NY: IBM Corp).

Results

Temperatures during surface activity away from burrow aprons

During surface activity away from burrow aprons outside rain events, mean shell temperature of hatchlings was 32.5 ± 3.7 °C (median = 32.6 °C, range = 23.6–43.6 °C, n = 578 temperature recordings during 92 activity bouts by 10 hatchlings; Figure 1a). Five hatchlings experienced shell temperatures ≥ 40 °C during at least one activity bout. Mean operative temperature on burrow aprons was 36.7 ± 6.3 °C (median = 36.0 °C, range = 24.5–57.5 °C, n = 242 temperature recordings during 82 activity bouts by 9 hatchlings; Figure 1b). Mean operative temperature in the open field, where the model was typically not shaded by vegetation, was 41.6 ± 7.4 °C (median = 42.5 °C, range = 27.0–58.0 °C, n = 190 temperature recordings during 73 activity bouts by 9 hatchlings; Figure 1c). All 9 hatchlings for which environmental operative temperatures were available engaged in activity away from burrows at least once when operative temperatures in the open field were ≥ 46 °C. Video observations indicated hatchlings routinely walked through sunlit areas when operative temperatures were high. Hatchling shell and environmental temperatures indicate that individuals engage in activity at temperatures where overturning on burrow aprons or in other sunlit areas could result in overheating in as few as 5 or 4 minutes, respectively (Figures 1d,e).

Overturning in the field

In the field, video cameras documented 15 naturally occurring overturning events involving 13 hatchlings. In 4 of 15 overturning events, hatchlings did not fully overturn because they caught themselves or landed with their carapace against the burrow

entrance. Individuals overturned when walking over small logs or uneven terrain associated with burrow entrances ($n = 11$ events; Figure 2); during combat with other hatchlings (2 events; Figure 2); for an unknown reason (1 event); and after being kicked, apparently inadvertently, by an adult tortoise (1 event). Because not all field overturning events were filmed at 1 frame per second, it was not possible to always resolve latency times to within 1 second. In 10–13 of 15 overturning events, individuals initiated active righting attempts within 1 second, and all hatchlings initiated righting within 6 seconds. Active righting time in the field varied, apparently reflecting variable time required to obtain grip to execute the righting maneuver, but in no instance was longer than 76 seconds. One hatchling partially overturned when local operative temperature exceeded CTM. When returning from activity away from its burrow, the hatchling ($T_{\text{shell}} = 37^{\circ}\text{C}$) partially overturned on its burrow apron ($T_e = 52.5^{\circ}\text{C}$). I estimate that, notwithstanding evaporative cooling mechanisms (e.g., voiding, frothing), this animal would have reached CTM in 5.5 minutes.

Laboratory righting experiment

In the laboratory experiment, righting success was much lower on loose sand than on structured sand (Mann-Whitney U test, $p < 0.001$). Only 2 of 12 hatchlings (2/60 trials) righted on loose sand, whereas 30 of 30 hatchlings (142/150 trials; Table 1) righted on structured sand. Regardless of substrate, tortoises usually required multiple attempts to obtain grip needed to execute the active righting maneuver. Temperature affected latency (Friedman test, $p < 0.001$; Figure 3) and active righting (Friedman test, $p < 0.001$; Figure 4) time. Latency times generally declined with increasing temperature (Figure 3). On

structured sand, active righting times exhibited a similar pattern (Figure 4). Latency times in the laboratory were longer than in the field (shortest latency times in the lab [32, 36 °C] vs. Field Max latency time: Mann-Whitney U tests, $p \leq 0.004$; Figure 3). Active righting time in the laboratory on structured sand at 32 and 36 °C did not differ from active righting time in the field (Mann-Whitney U tests, $p \geq 0.131$; Figure 4).

Discussion

Similar to some other turtles, hatchling gopher tortoises overturned when negotiating uneven terrain, including small logs and back edges of burrow entrances, and during aggressive conspecific interactions (Hosier et al. 1981, Steyermark and Spotila 2001, Limpus et al. 2003, Radzio et al. 2016; Chapter 5). Most overturning events occurred in association with activity away from burrows. During such activity, wild hatchlings fitted with temperature loggers exhibited moderate to high shell temperatures, centered around the preferred body temperature of fed hatchlings (Johnston 1996; Chapter 2), and often moved through open sunlit patches where operative temperatures exceeded the species' critical thermal maximum (43.9 °C, Hutchison et al. 1966). Thus, by engaging in locomotor activity in hot environments, hatchlings voluntarily expose themselves to some degree of overheating risk associated with overturning. Given their small mass and low thermal inertia, hatchlings that overturn in hot patches must right quickly to avoid thermal stress. Emergency responses such as frothing and bladder voiding could stave off extreme overheating via evaporative cooling (Douglass and Layne 1978, Johnston 1996), but only temporarily.

Despite high temperatures, the field observations indicate that overturning in a hot patch would typically not place a hatchling at great danger of overheating because individuals usually right quickly. In the field, overturned hatchlings required no more than 76 seconds to complete the active righting maneuver, including tortoises fitted with miniature temperature data loggers and/or radio transmitters, which potentially hinder righting. Although hatchlings righted quickly in the field, several individuals required many attempts to do so. Thus, had I observed a larger number of field overturning events, I may have documented atypical instances where hatchlings required substantially longer to right. Rare instances where individuals may be unable to right quickly may play an important role in shaping turtle behavior because failure to right fast in a hot patch can result in death. Alternatively, if individuals are only very rarely unable to right before overheating, benefits of activities that can result in overturning in hot environments, potentially including reduced predation risk associated with foraging during midday hours (Johnston 1996), may outweigh costs.

The laboratory experiment suggests hatchlings are unable to right quickly enough on loose sands to avoid reaching CTM in very hot conditions. Though not very common in most tortoise habitats, tortoises can encounter loose sands on burrow aprons, in coastal dune environments, and on sand roads (Pawelek and Kimball 2014, Gilson and Bateman 2015; Radzio personal observation). Gilson and Bateman (2015) reported a dead, but intact tortoise overturned in a tire rut of a sand road at Archbold Biological Station and concluded that it likely died of exposure and that deep tire ruts can be barriers to tortoise movement (Hosier et al. 1981). Additional observations, including field righting

experiments, are warranted to understand how gopher tortoises interact with loose sands, particularly in habitats where this substrate is common (e.g., coastal dunes).

Hatchlings of some species exhibit variable and rather long latency times in experiments where individuals are manually overturned by a researcher, perhaps reflecting prioritization of antipredator responses over righting immediately (e.g., Steyermark and Spotila 2001, Polo-Cavia et al. 2012, Davy et al. 2014, Mitchell et al. 2016). In this study, latency times were more variable and longer in the laboratory experiment than in the field. Whether turtles commonly exhibit long latency times in nature is unknown as investigations of turtle responses to naturally occurring overturning events are rare. The low but finite probability that an individual may need a long time to obtain grip required to right may be one driver of short latency times in hatchling gopher tortoises. Additionally, the earlier individuals right, the sooner they regain the availability of one of their most important antipredator tactics, quickly hiding inside burrows (Wilson et al. 1994, Mushinsky et al. 2003, Radzio and O'Connor 2017; Chapter 3). In my study population, hatchling and juvenile gopher tortoises limit most basking to burrow aprons and are highly vigilant, such that they typically flee into burrows before they can be observed by an approaching researcher (Radzio and O'Connor 2017; Chapter 3).

Consistent with most available studies of other turtles, active righting times in the laboratory generally decreased with increasing temperature (Elnitsky and Claussen 2006, Ben-Ezra et al. 2008, Polo-Cavia et al. 2012), likely reflecting thermosensitivity of physiological performance (Bennett 1980) and/or greater motivation to right at elevated body temperatures. However, individuals maintained a high degree of righting performance across a wide range of temperatures. This can be important because,

although hatchlings experience high shell temperatures during typical surface activity away from burrow aprons outside of rain events, young individuals can experience low body temperatures away from burrows during rainstorms, when they drink water that temporarily accumulates on the ground (Radzio and O'Connor, unpublished data). Temperature effects on active righting response may also provide insights into the thermosensitivity of other locomotor performances. This could include rapid movements into burrows to avoid potential predators (Radzio and O'Connor 2017; Chapter 3), which young individuals routinely perform at low body temperatures when basking on burrow aprons in early spring (Radzio and O'Connor, unpublished data).

Short latency and active righting times documented in this study appear well suited for hatchling tortoises that are regularly active in hot environments, navigate uneven terrain, and engage in combat with conspecifics (Butler and Sowell 1996, Radzio et al. 2016). Although I only measured righting performance at temperatures up to 36 °C in the laboratory, the field observations indicate that it is likely important for individuals to maintain righting performance at substantially higher temperatures. The fact that tortoises returning to burrows with shell temperatures ≥ 40 °C exhibited normal, coordinated locomotion (Radzio and O'Connor, unpublished data) may provide insights into righting capacity at high body temperatures, but shell temperatures may overestimate internal body temperatures (Kornilev et al. 2006). Future work could assess effects of thermal environment on the timing and duration of hatchling foraging bouts, internal body temperatures during foraging activity, righting performance at those temperatures, and whether overheating risk associated with overturning influences how individuals

negotiate physical obstacles (Golubović et al. 2013a; e.g., crawl over rather than around logs).

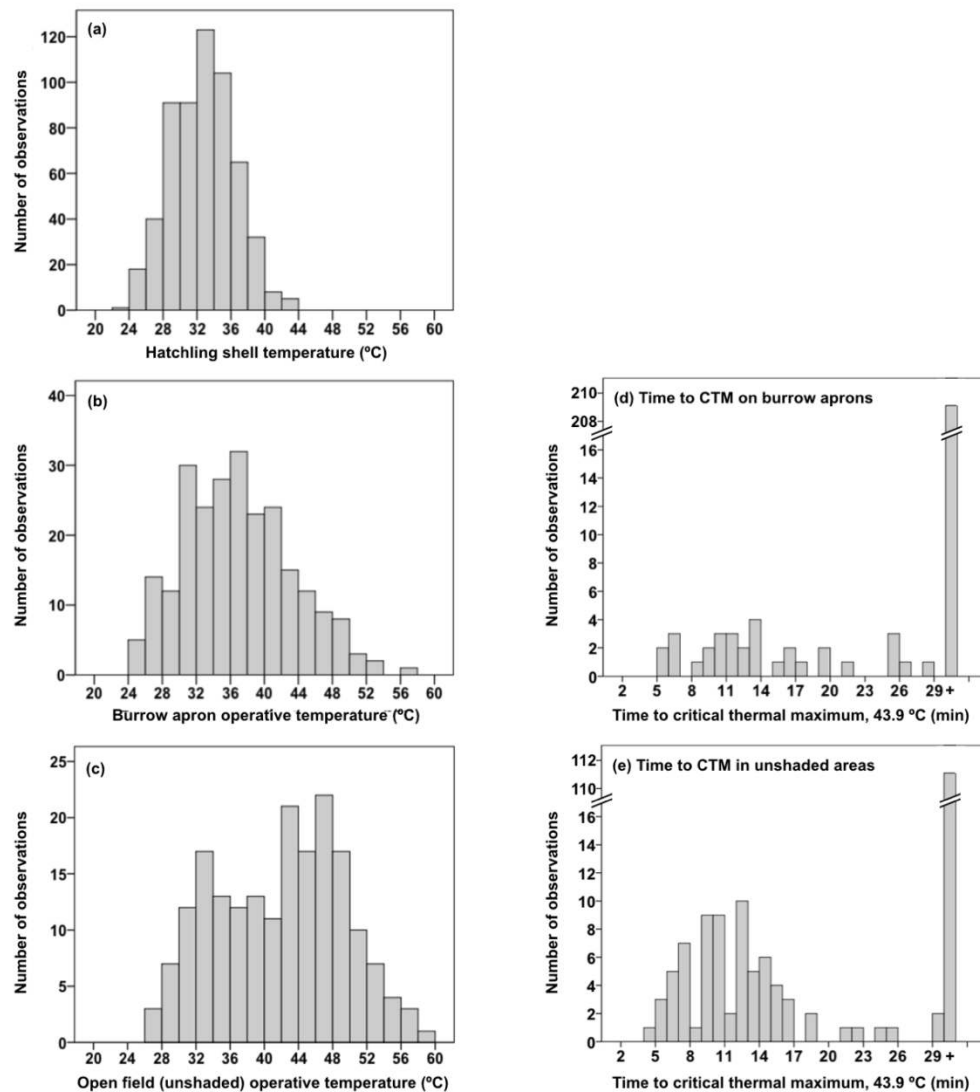


Figure 0-1. Shell temperatures during surface activity away from burrow aprons for ten hatchling gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA (a). Corresponding, operative temperatures on burrow aprons (b) and in an open field (c) during surface activity away from burrow aprons for nine hatchlings and how long it would take overturned individuals to reach CTM at burrow apron (d) or open field (e) operative temperatures, estimated for all time-paired shell and environmental temperature recordings.

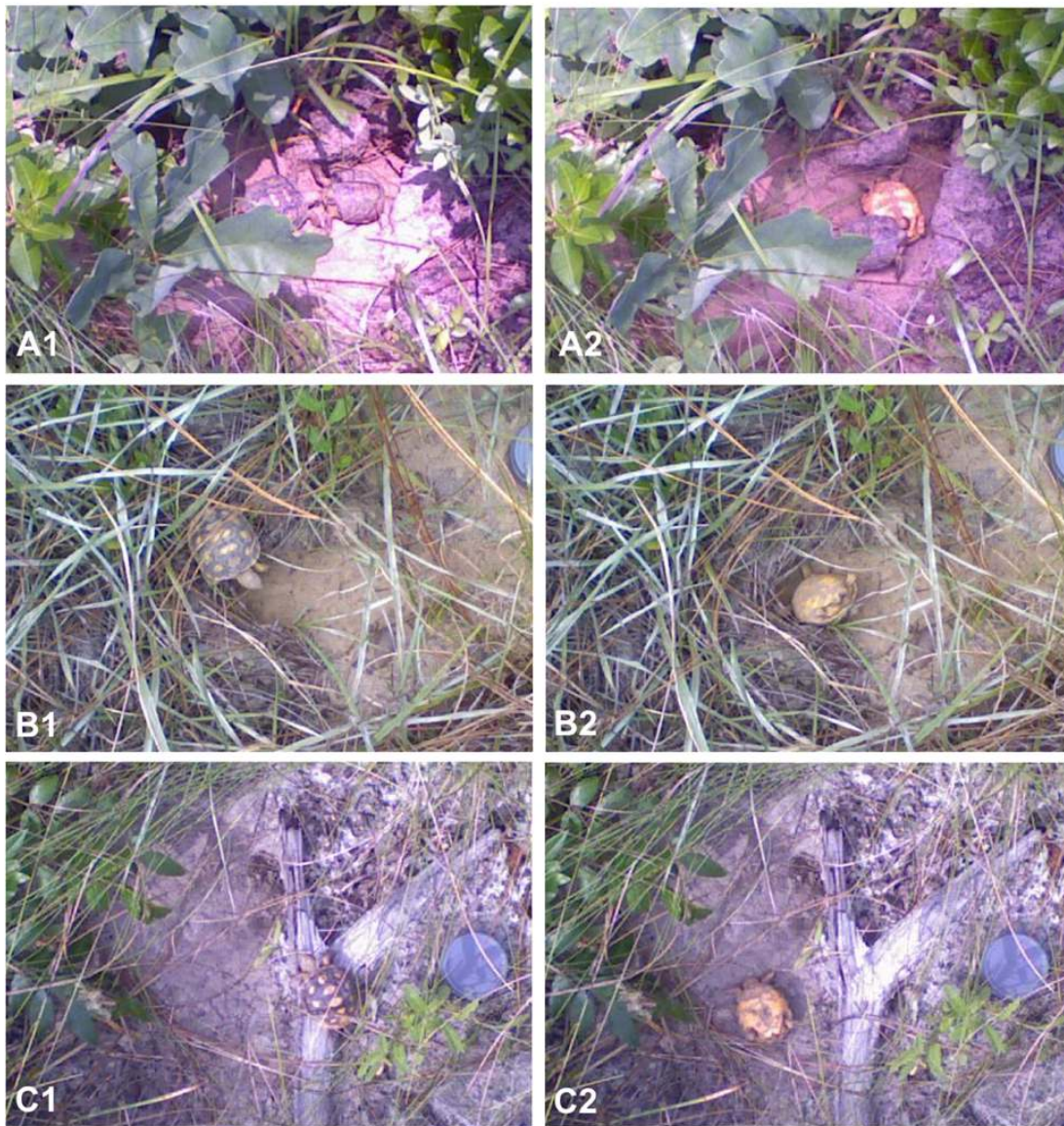


Figure 0-2. Examples of naturally occurring overturning events for free-ranging hatchling gopher tortoises inhabiting a longleaf pine forest in southwest Georgia, USA. A1–A2: Hatchling-hatchling combat. B1–B2: Approaching burrow entrances from behind. C1–C2: Crawling over small logs.

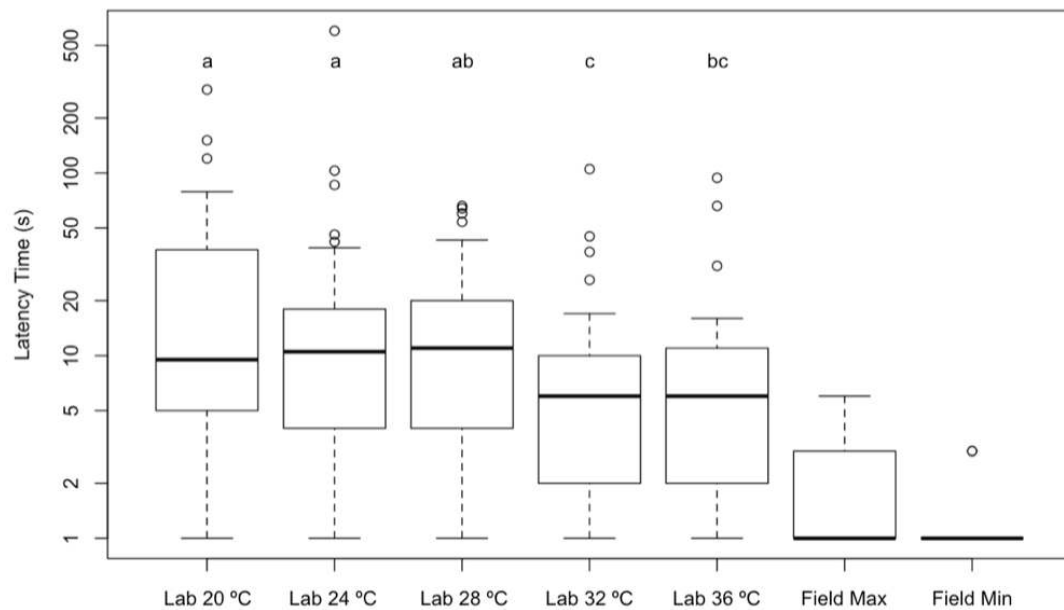


Figure 0-3. Latency times (defined in Methods) of hatchling gopher tortoises in laboratory righting trials at 5 temperatures ($n = 42$) and of free-ranging hatchlings in response to naturally occurring overturning events in the field documented by video cameras at tortoise burrows ($n = 13$). I calculated minimum (Field Min) and maximum (Field Max) latency times for free-ranging hatchlings because in some instances cameras were programmed to record a frame every 6 seconds, rather than once per second as in the laboratory. Thick horizontal bars inside boxes denote medians; boxes denote interquartile ranges; whiskers denote $1.5 \times$ interquartile ranges; and circles denote values falling outside $1.5 \times$ interquartile ranges. Laboratory temperature treatments that do not share at least one letter (above box plots) are significantly different from each other (pairwise Wilcoxon signed-rank tests with Bonferroni adjustments). Field Max latency time was significantly shorter than the shortest latency times in the laboratory (occurring at 32 and 36 °C, Mann-Whitney U test, $p \leq 0.004$).

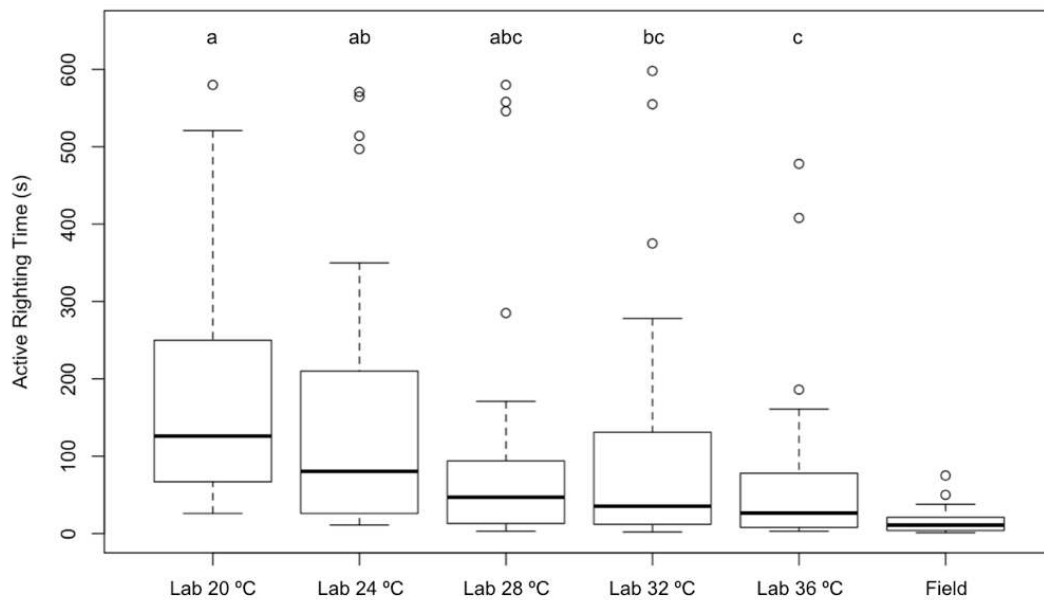


Figure 0-4. Active righting times (defined in Methods) of hatchling gopher tortoises on structured sand in laboratory righting trials at 5 temperatures ($n = 30$) and of free-ranging hatchlings in response to naturally-occurring overturning events in the field ($n = 13$). See Figure 4-3 caption for box plot definitions. Laboratory temperature treatments that do not share at least one letter (above box plots) are significantly different from each other (pairwise Wilcoxon signed-rank tests with Bonferroni adjustments). Active righting time in the field did not differ from active righting time in the laboratory on structured sand at 32 or 36 °C (Mann-Whitney U test, $p \geq 0.131$). Plotted field active righting times include partially and fully overturned individuals ($n = 13$), but field versus laboratory statistical comparisons are performed using only animals that fully overturned ($n = 9$).

Table 0-1. Fraction of hatchling gopher tortoises that righted during 10-min laboratory trials on loose (n = 12 hatchlings) or structured sand (n = 30 hatchlings) at five test temperatures.

Substrate / Temperature	20 °C	24 °C	28 °C	32 °C	36 °C
Loose Sand	0/12	0/12	0/12	1/12	1/12
Structured Sand	28/30	28/30	28/30	28/30	30/30

CHAPTER 5: AGGRESSION, COMBAT, AND APPARENT BURROW COMPETITION IN HATCHLING AND JUVENILE GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*)

Introduction

Aggression can enhance an individual's ability to compete for resources or mates and features prominently in the ecology of many turtle species, including North American tortoises (*Gopherus* spp.). For example, adult male desert (*Gopherus agassizii*) and gopher (*Gopherus polyphemus*) tortoises engage in combat (i.e., repeated ramming, flipping) with other males, presumably because it increases access to reproductive females (Auffenberg 1977, Niblick et al. 1994). In another context, female desert tortoises and, sometimes, gopher tortoises defend nests against potential predators (Barrett and Humphrey 1986, Zylstra et al. 2005, Gienger and Tracy 2008, Grosse et al. 2012, Agha et al. 2013, Dziadzio and Smith 2015). Adult gopher tortoises also aggressively compete with conspecifics for burrows (Douglass 1976, McRae et al. 1981, Diemer 1992), a key resource that requires lengthy investments of time to construct and is used extensively for thermoregulation, predator avoidance, and other important activities (e.g., sleeping, mating, and nesting; Douglass and Layne 1978, Landers et al. 1980, Johnson et al. 2007).

Although aggression is a common characteristic of North American tortoises, most observations of aggression in this group, and turtles in general, involve mature individuals. Immature tortoises do not compete for mates or defend nests and are generally considered docile. Immature gopher tortoises do, however, occasionally cohabitate burrows (McRae et al. 1981, Diemer 1992, Butler et al. 1995, Pike and

Antworth 2005), and this proximity provides a setting where agonistic interactions might be predicted if, similar to adult tortoises, immature tortoises also compete for burrows. Diemer (1992) reports an instance of burrow competition between two “subadult” (large, nearly mature) gopher tortoises. Adult-like behaviors might be expected in animals approaching maturity, but an observation by Butler and colleagues (1995) suggests that hatchlings also aggressively defend burrows. The authors report “a hatchling ramming another who had encroached on the defender’s burrow apron”, but did not share additional information about the encounter. Aside from this account I am unaware of other reports of aggression in hatchling or small juvenile gopher tortoises.

It is unclear whether aggression is common in young gopher tortoises because few studies have focused on interactions among immature individuals of this species. Young gopher tortoises are difficult to observe, in part because they tend to retreat into burrows quickly when approached (Wilson et al. 1994, Radzio and O’Connor 2017, Chapter 3). In a detailed study of juvenile gopher tortoise ecology, Wilson et al. (1994) reported on the activity budgets and movements of young tortoises fitted with radio transmitters but did not include information regarding social behaviors. Pike and Antworth (2005) presented several observations of burrow cohabitation without overt aggression but also identified the need for future investigation focusing on potential social interactions in hatchling and juvenile gopher tortoises.

I used time-lapse video cameras to study the behavior of young gopher tortoises at a site in southwest Georgia. My observations included several instances of aggressive interactions with behaviors such as ramming and flipping that are well known for adult

gopher tortoises. Here, I describe these aggressive interactions and discuss their potential functions in the ecology of hatchling and juvenile gopher tortoises.

Materials and Methods

I conducted this study on Arcadia Plantation (30°45'N, 84°0'W), located in Thomas County in southwest Georgia. The study site was dominated by mature (> 80 years) longleaf pine (*Pinus palustris*) forest and included the Wade Tract, an 80-ha research area that has never been heavily cut and contains many old trees (> 200 years; Platt et al. 1988). Ground cover on both Arcadia Plantation and the Wade Tract was dominated by wiregrass (*Aristida stricta*), oaks (*Quercus* spp.), and a diverse assemblage of other native plants (Ambrose 2001). Soils at the site are a complex mosaic of sand and clay (NRCS Web Soil Survey 2016). The study area was managed using frequent prescribed fire (< 2-year return intervals).

During June to September 2011, December 2011 to January 2012, March to September 2012, December 2012, April to October 2013, and September to October 2014, I studied hatchling and juvenile gopher tortoise thermoregulatory and antipredator behavior at the site. Observations of tortoise interactions were incidental to these studies and were made using time-lapse cameras (primarily Plotwatcher Pro, Day 6 Outdoors, Inc., Columbus, GA) that recorded tortoise activity at neonate (age, 0.0–0.5 year), hatchling (age, 0.5–1.0 year), and juvenile burrows continuously (usually one frame per second) during daylight hours. For some of this research (e.g., thermoregulatory movements), I avoided recording at burrows known to contain multiple tortoises, and for other research, I specifically filmed burrows known to

contain multiple tortoises to observe interactions. Because of this nonrandom collection of video data, I did not estimate burrow sharing frequency. All observations of tortoise interactions were of wild animals. Interactions involving individuals reared in the laboratory and subsequently released as yearlings back into the site (in September 2014) were not included in this study.

I scored a tortoise as aggressive if it rammed or repeatedly pushed another individual (Weaver 1970, Auffenberg 1977, Ruby and Niblick 1994). I also documented other agonistic behaviors such as biting, flipping, and withdrawal (Weaver 1970, Auffenberg 1977, Ruby and Niblick 1994). When possible, I identified unique individuals by size, location within the study site, or presence of radio transmitters or miniature iButton temperature loggers (Maxim Integrated Products, Sunnyvale, CA) affixed to shells (used to study thermoregulatory and antipredator behavior at the site). Three hatchlings (3L10R, 3R10R, 10R) and two juveniles (2R3L, 330.630) documented to engage in overt aggression had radio transmitters and/or iButtons attached to their carapaces at the time of observations.

Results

Tortoise Encounter Summary and Aggression

Video cameras documented hatchling or juvenile tortoises cohabitating (sharing a burrow overnight) in 5 burrows. Cameras also documented hatchlings or juveniles briefly visiting 8 burrows already occupied by an immature conspecific without sharing the burrow overnight. These encounters, observed at 12 different burrows (one burrow was both a cohabitation and brief visitation site), usually involved a pair of

tortoises, but in 2 instances, 3 immature tortoises cohabitated a burrow. Cameras documented tortoises cohabitating up to 8 consecutive days, but tortoises may have cohabitated longer because cameras did not record the beginning and end of each cohabitation event.

The presence of a second or third tortoise in a burrow hindered free movement of occupants in and out of burrows in each of five observed instances of burrow cohabitation. Qualitatively, burrow dimensions (widths and heights) were similar to those of resident tortoises, making it difficult or impossible for individuals to pass one another in the burrow shaft.

Cameras documented aggression in 6 of 13 (46%) instances of cohabitation or brief visits to occupied burrows. In total, cameras recorded 4–6 (some tortoises were unmarked) individual hatchlings and 5 individual juveniles exhibiting overt aggression toward another immature tortoise. In 3 brief visits (< 8 minutes) where aggression was not observed, the visiting tortoises only looked inside the occupied burrow entrance or briefly entered before leaving. These tortoises may have left in response to the presence of the resident tortoise. However, some tortoises cohabitated burrows for many days, without engaging in overt aggression.

Hatchling–Hatchling Aggressive Interaction 1

The most dramatic instance of aggression involved 2 hatchlings (age, ~9–10 months), and included repeated pushing, apparent biting, and flipping (Figure 1). At 1118 hrs on 2 June 2013, the resident hatchling (ID: 3L10R, carapace length = ~61 mm, mass = ~57 g) left its burrow apron, presumably to forage. At

1145 hrs, a visiting hatchling (ID: 3R10R, carapace length = ~61 mm, mass = ~66 g), whose burrow was located ~4 m away, arrived at the resident's burrow and entered. At 1148 hrs, the resident hatchling returned and immediately entered its burrow, which still contained the visitor. At 1223 hrs, the resident hatchling reemerged, and 2 min later the visitor appeared in the burrow entrance behind the resident, repeatedly extending its head toward the resident, possibly biting at an iButton temperature logger attached to the resident's rear vertebral scute. At 1226 hrs, the resident turned around and faced the burrow entrance and the visitor (who was still in the entrance). Both hatchlings immediately began to push against each other with their forelimbs and gular protrusions, keeping their heads withdrawn during the exchange (Fig. 1A). The opposing forces of the tortoises' (bodies) caused their anterior plastrons to rise off the ground (Fig. 1A). This continued for several seconds until the visitor (still in the burrow entrance) flipped the resident onto its carapace (Fig. 1B–C).

After being overturned, the resident hatchling required 50 sec to right itself. Both then remained motionless for an additional 40 sec. Next, the resident turned toward the visitor (which faced outward from the burrow), withdrew its head, and used its gular protrusion, forelimbs, and carapace to ram the visitor against the entrance until it was pinned in a nearly vertical orientation (Fig. 1D–F). Both tortoises then resumed pushing and biting each other with their heads retracted until the visitor retreated into the burrow. The resident remained at the surface facing the burrow.

About 3 min later, the visitor reemerged and advanced toward the resident until the resident moved backward one body length. Following this movement, the resident charged the visitor, once again pushing it backward into a vertical orientation against the burrow entrance. The resident repeatedly rammed the visitor until the visitor pushed past the resident and returned to its own burrow nearby (observed by another camera located at the visitor's burrow). The resident continued to bask in front of the burrow for several more minutes before entering inside.

Hatchling–Hatchling Aggressive Interaction 2

Around midday on 26 June 2012, I briefly placed a metal tablespoon inside a hatchling burrow. Shortly after holding the spoon in the entrance, an unmarked hatchling started to repeatedly ram the spoon and continued to do so as the spoon was pulled slowly out of the burrow. The hatchling was captured as it followed the spoon out of the burrow, and a second hatchling (unidentified) emerged from the burrow within seconds, apparently in response to the exit of the first individual. I was unable to capture the second tortoise before it reentered the burrow.

Shortly before 0900 hrs on the following day, I returned the hatchling (still unmarked) to its burrow (which still contained the other unidentified hatchling) and set a camera to monitor interactions. The camera first observed several hatchling movements in and out of the burrow without overt aggression. Aggression was apparent starting at 1131 hrs, when one hatchling attempted to reenter the burrow, but was unable to do so because the other tortoise occupied the entrance.

Although it was difficult to interpret from the video, it appeared that as the outermost hatchling attempted to reenter the burrow, the hatchling in the entrance pushed back several times and prevented it from entering. After a few minutes, the hatchling in the entrance went deeper into the burrow and the outermost hatchling entered the inside edge of the burrow. The outermost hatchling reemerged to bask again at 1138 hrs. At 1148 hrs, the outermost hatchling again turned to face the entrance as if attempting to enter, but the other hatchling blocked the entrance and appeared to push the outermost hatchling backward as it attempted to enter. After unsuccessfully attempting to reenter the occupied burrow, the outermost hatchling left the area at 1152 hrs.

Hatchling–Hatchling Aggressive Interaction 3

At 1040 hrs on 3 August 2012, an unmarked hatchling tortoise approached a burrow while the marked resident hatchling (ID: 10R) was inside. The visiting hatchling attempted to enter the burrow, at which point the resident hatchling (now in the burrow entrance) repeatedly pushed back against the visitor. This sequence repeated itself several times. Because the resident hatchling was in the entrance during the entire encounter, it was not possible to observe additional details. At 1048 hrs, the visiting hatchling left the area. Hatchling–hatchling aggressive interactions 2 and 3 occurred several meters apart and, therefore, could have involved the same individuals (the one marked hatchling [10R], was marked after interaction 2 occurred).

Hatchling–Juvenile Aggressive Interaction 1

At 1134 hrs on 7 August 2013, a juvenile tortoise (ID: 2R3L, age = 2 yrs, carapace length = ~83 mm) was inside its burrow when a hatchling tortoise (age, ~11 mo) visited and entered just inside the burrow. About 25 sec after entering the burrow, the hatchling turned around and faced out of the burrow entrance. Less than a minute later, the hatchling emerged fully with the larger juvenile repeatedly pushing it from behind. The juvenile had its head withdrawn into its shell as it pushed the hatchling, suggesting that it was responding aggressively toward the visitor. Once on the burrow apron, the hatchling turned around to face the juvenile before leaving the area soon thereafter at 1136 hrs.

Juvenile–Juvenile Aggressive Interactions 1 and 2

At 1034 hrs on 29 July 2012, a juvenile tortoise (ID: 330.630, age = ~9 yrs, carapace length = 143 mm) that was basking in front of its burrow rapidly entered inside. A few seconds later, another slightly larger juvenile (unmarked) arrived at the burrow, stopped just before the entrance, and faced inside. At 1039 hrs, the visitor advanced toward the burrow, and the resident juvenile emerged to the entrance and repeatedly rammed the intruder, which retreated backward about three-quarters of a body length. The two tortoises sat motionlessly facing each other for about 2 min. Then, the resident aggressively advanced toward the visitor, which responded by backing up to the edge of the burrow apron, where the aggressive interaction continued under some vegetation and mostly out of camera view. At 1043 hrs, the resident quickly backed into its burrow while the visitor remained at

the edge of the burrow apron. From 1043 to 1045 hrs, the visitor advanced incrementally toward the burrow entrance until it partially entered the burrow at 1053 hrs. The resident was still inside the burrow. Then at 1058 hrs, both tortoises started repeatedly ramming each other head-on with their heads withdrawn into their shells. At 1105 hrs, the larger visiting tortoise left the area.

Cameras documented a second aggressive interaction involving 2 different unmarked juvenile tortoises (~6-yr-olds, age estimated from video). The two tortoises were inside a burrow when the camera started recording at 0708 hrs on 3 September 2014. Also present inside the burrow was a third tortoise, a yearling that was raised in the laboratory and later released back into the site on 1 September 2014. Only the yearling tortoise emerged from the burrow on 3 September. On the following morning, the yearling left the burrow area without returning, and the two larger juveniles emerged shortly thereafter. They moved between the burrow and surface several times without overt aggression but shortly before 1500 repeatedly pushed each another back and forth in the burrow entrance before entering inside for the remainder of the day.

Additional Aggressive Interactions Between Tortoises and Objects

I observed 2 additional tortoises (1 hatchling and 1 juvenile) respond aggressively toward objects placed inside or near burrows. Around midday on 7 June 2013, I attempted to remove an iButton miniature temperature logger from 10 cm within hatchling 3R10Rs (visitor from Interaction 1) burrow using a pair of long-nosed pliers. Hatchling 3R10R advanced toward the pliers. Later, I wiggled a (~1.5 cm in diameter) dowel rod in the burrow to simulate the bobbing head of a

visiting tortoise. The hatchling repeatedly rammed the dowel as if it was confronting an intruder. The hatchling responded in the same manner to multiple insertions of the dowel into its burrow. Although it advanced toward the burrow entrance as I slowly removed the dowel, it did not fully emerge. On 28 July, 30 July, and 4 August 2013, a camera documented a 3-yr-old juvenile (3R3L) repeatedly ramming/pushing, with its head partially withdrawn, an operative temperature model that had been placed earlier on the burrow apron. The grey model was of similar size, shape, and color of a tortoise but did not have a head or limbs. I did not document any other tortoises clearly interacting with models. In all of the above examples, the vigor of the tortoises' interactions with the objects (spoon, dowel rod, or temperature model) indicated aggressive behavior.

Discussion

Although infrequent and difficult to observe, aggression likely plays an important role in the ecology of hatchling and juvenile gopher tortoises by influencing access to burrows. Young tortoises come into contact with one another at burrows where they either respond aggressively or cohabitate for prolonged periods without overt aggression (Butler et al. 1995). Varied responses to conspecifics may reflect individual or situational differences but may also be an artifact of the fact that cameras did not document activities inside burrows where tortoises spent most of their time. Therefore, some individuals may have engaged in unobserved agonistic interactions in burrows. Notably, the observed aggressive behaviors were remarkably similar to those of adult *Gopherus* and included repeated ramming, pushing, and

flipping (Auffenberg 1977, Niblick et al. 1994, Ruby and Niblick 1994). Hatchlings also sometimes bit each other during these exchanges.

Hatchling and juvenile tortoises may respond aggressively to conspecifics because the presence of a second individual inside a burrow interferes with thermoregulatory and antipredator movements. Tortoise burrows are not much wider than the resident tortoise, making it difficult or impossible for individuals to pass one another in the burrow shaft, which even in the case of juvenile tortoises, can be several meters long. Young gopher tortoises at the study site basked extensively just in front of their burrows and made frequent movements in and out of burrows, presumably to regulate body temperature (Chapter 2). The presence of a conspecific hindered such movements. Hatchlings and juveniles cannot remain at the surface in open habitats for extended periods during hot weather (operative temperatures at the site frequently exceed 50 °C), and aggressive interactions at burrows can affect the ability of a hot individual to escape into an occupied burrow before overheating.

Burrow cohabitation may hinder antipredator movements in young tortoises. Hatchling and small juvenile gopher tortoises have soft shells and are vulnerable to many predators, including snakes, mammals, and birds (Fitzpatrick and Woolfenden 1978, Butler and Sowell 1996, Epperson and Heise 2003, Stevenson et al. 2010, Stevenson 2014). In my study population, basking tortoises (hatchlings, juveniles, and adults alike) usually retreat rapidly into burrows when approached (Radzio and O'Connor 2017, Chapter 3). However, I routinely observed instances in which the presence of a tortoise just inside a burrow prevented another individual from entering

deeper inside. Often the outermost tortoise was seen at the burrow entrance frantically pushing on the tortoise that was deeper in the burrow in an effort to enter further inside. Sharing burrows may place individuals that are slower to enter burrows at greater predation risk than individuals occupying burrows alone.

Young tortoises do not always respond aggressively to conspecifics that visit their burrows, perhaps because aggressive behaviors involve energy expenditure and carry some risk. As I document, hatchlings may flip one another during combat, and it is conceivable that in certain situations a young tortoise may be unable to right itself before overheating. For example, the flipped tortoise in my observation (Interaction 1) required nearly 50 sec to right itself, which is similar to the righting time required by other hatchlings I have observed overturned in the field (Chapter 4). However, hatchlings may not be able to right themselves in loose sand if there is no vegetation or structure nearby to provide the grip required for the righting maneuver (Chapter 4). Additionally, aggression that involves biting may result in injuries. I observed this firsthand when I placed two hatchlings together in a container in the laboratory as part of other work. In that case, one individual bit the other, producing a scratch just next to the eye. Finally, my observations of hatchlings repeatedly ramming both dowel rods and metal spoons placed inside burrows and advancing to burrow entrances or even fully emerging in response to foreign objects, suggest that similar misplaced aggression toward a predator could result in increased predation risk.

The accounts presented here, along with that of Butler et al. (1995), indicate that hatchling and juvenile gopher tortoises exhibit a wider range of social behaviors than previously recognized. Aggressive behaviors of immature tortoises are similar

to those of adult conspecifics, and probably play a role in determining burrow use patterns, particularly as young tortoises come into contact with conspecifics at burrows. I conclude that, although ecologically important, such interactions are probably underreported, perhaps in large part because of the wary nature of immature gopher tortoises and their tendency to quickly hide in burrows when approached. However, time-lapse cameras provided windows into the activities and behaviors of young tortoises, including their social interactions. Future studies could examine potential costs associated with burrow cohabitation (e.g., constraints on thermoregulation and predator avoidance), individual variation in aggressive tendency, factors that may underlie such potential variation (e.g., personality, relatedness, age, sex), and how aggressive interactions influence patterns of burrow use and cohabitation in young gopher tortoises.



Figure 0-1. Combat between a resident (right in all frames) and visiting (left) hatchling (age = ~9–10 month) gopher tortoise on 2 June 2013 at Arcadia Plantation in southwest Georgia. (A–C) The visiting tortoise flipped the resident onto its carapace; (D–F) upon righting itself, the resident pushed the visitor into a vertical orientation against the back of the burrow entrance; (G–I) following additional interactions (described in the text), the resident again pushed the visitor into a vertical orientation against the burrow entrance. Shortly thereafter (not shown) the visiting tortoise left and returned to its burrow, suggesting that aggressive interactions may limit burrow sharing by hatchling tortoises.

CHAPTER 6: BEHAVIOR, GROWTH, AND SURVIVAL OF LABORATORY-REARED GOPHER TORTOISES FOLLOWING HARD-RELEASE

Introduction

Maintaining animals in captivity constitutes a vital component of many conservation and research programs (Swimmer et al. 2005; Crone et al. 2007; Griffiths and Pavajeau 2008). However, releasing captive animals into the wild can present challenges for practitioners because released individuals often experience high predator-induced mortality (Moseby et al. 2011; Holbrook et al. 2015; Tuberville et al. 2015). Released animals can be more vulnerable to predators than their wild counterparts for various reasons, including because they are unfamiliar with their surroundings, have yet to establish refugia, and may exhibit unnatural, deleterious behaviors (Beck et al. 1994; Tuberville et al. 2015). Measures to improve reintroduction success include captive conditions that promote natural behavior and release protocols that reduce interactions with predators (Biggins et al. 1998; Stamps and Swaisgood 2007; Reading et al. 2013; Tuberville et al. 2015).

Turtles are declining worldwide owing to a variety of threats (Buhlmann et al. 2009; Böhm et al. 2013). For some critically endangered turtle species, captive breeding represents a necessary action to maintain or reestablish viable wild populations (Gibbs et al. 2014; Spencer et al. 2017). Captive breeding can work, but success may require decades of repeated effort (Gibbs et al. 2014), in part due to naturally low survivorship of small juvenile turtles (Congdon and Gibbons 1990; Heppell et al. 1996). Long-term evaluations may be needed to truly understand the effectiveness of such programs (Dodd

and Seigel 1991; Gibbs et al. 2014); however, short-term studies of behavior, growth, and survival following release can provide important information for intermediate assessment and periodic recalibration of release efforts (Pedrono and Sarovy 2000; Lepeigneul et al. 2014; Tuberville et al. 2015; Spencer et al. 2017).

Development of release protocols and understanding of captivity effects on behavior is not only relevant for conserving critically endangered turtles, but can also be beneficial in situations where other management measures may be favored. For example, studies of released juvenile turtles can provide valuable insights into the ecology, including habitat requirements, of a life stage that is poorly understood in most chelonians (e.g., Mansfield et al. 2012; Nagy et al. 2015; Todd et al. 2016; Nafus et al. 2017). However, such studies depend on the assumption that behavior does not differ materially between released and wild individuals. Additionally, established release protocols can enable researchers to responsibly and efficiently release captive turtles at the conclusion of experiments, which can be particularly important when working with sensitive species.

The gopher tortoise is a threatened herbivore of the southeastern United States (McCoy et al. 2006; Smith et al. 2006; USFWS et al., 2013) that is important ecologically because the long burrows it constructs are used by many other animals, including several obligate commensals (Jackson and Milstrey 1989; Pike and Mitchell 2013; Dziadzio and Smith 2016). Individuals use burrows for thermoregulation and predator avoidance and spend most of their time inside these refugia (Douglass and Layne 1978; Wilson et al. 1994; Pike and Grosse 2006; Radzio and O'Connor 2017). Conservation priorities for the gopher tortoise include protection and restoration of well-drained upland habitats,

particularly longleaf pine (*Pinus palustris*) forests and savannas and translocation of animals from poor to higher quality habitats (Diemer 1986; Smith et al. 2006; Tuberville et al. 2008; USFWS et al., 2013). Biologists in several states are also investigating the management value of headstarting, a process where hatchlings are reared in captivity and released in high quality habitats as larger individuals when they are presumably less vulnerable to predators (Tuberville et al. 2015; Quinn et al. 2016).

Release methods for juvenile gopher tortoises, either animals raised in captivity for headstarting or for research purposes, include procedures where individuals are simply released without additional measures (i.e., hard release), and approaches where individuals might be provided starter burrows, penned to limit movements, or caged to limit movement and interactions with predators (i.e., soft release; Holbrook et al. 2015; Tuberville et al. 2015; Quinn 2016). Hard release represents the least expensive and time consuming method for releasing young gopher tortoises, but its effectiveness has not been assessed. If hard release could be demonstrated effective, it could reduce costs associated with the release of captive-reared tortoises in headstarting projects, as well as cases where young tortoises are released into the wild following laboratory research (Holbrook et al. 2015). Similarly, if released juvenile gopher tortoises exhibited normal behavior, they could be used to investigate conservation-relevant aspects of the species' biology, including habitat selection and survival.

I provide the first account of juvenile gopher tortoise responses to hard release. I collected gopher tortoise hatchlings as they emerged from nests and maintained individuals in a laboratory setting for the first year of their lives for physiology experiments (Chapters 2 & 4). At the conclusion of the experiments, I hard-released

tortoises as yearlings back on the site where they had been collected. I assessed burrowing behavior following release, including whether, like wild juveniles, released yearlings disproportionately construct burrows under deadwood (Aresco 1999). I also quantified released yearling activity, microhabitat use, and antipredator responses. I was particularly interested whether, similar to wild individuals, released tortoises spent most of their time in or near burrows, basked extensively, and hid in burrows when approached (Wilson et al. 1994; Pike and Grosse 2006; Radzio and O'Connor 2017; Chapters 2–3). Finally, I determined growth rates and apparent survivorship and evaluated these with respect to values for wild juveniles.

Materials and Methods

Study site

I conducted this study on Arcadia Plantation (30°45'N, 84°0'W), a 957-ha private property in southwest Georgia, USA. The site primarily consisted of mature and old-growth longleaf pine forest with ground cover composed predominantly of wiregrass (*Aristida stricta*), oak (*Quercus* spp.), and other native plants (Platt et al. 1988). Soils were a complex mosaic of sand and clay. The site was well managed using frequent prescribed fire and supported a large gopher tortoise population (Tuberville et al. 2014).

Tortoises

I collected hatchling tortoises in September–October 2013 as they emerged from natural nests (described in Radzio et al. 2017). Individuals were uniquely marked by notching scutes (Cagle 1939) and reared in a laboratory setting for thermal physiology experiments. Tortoises were housed indoors in small plastic enclosures, usually had

access to an artificial burrow, and were primarily fed organic chicory (*Chicorium sp.*), orchard grass, and Natural Grassland Tortoise Food (Zoo Med Laboratories, San Luis Obispo, California, USA), occasionally supplemented with calcium and vitamins (Herptivite, Rep-Cal Research Labs, Los Gatos, California, USA).

During most of their time in captivity, hatchlings were kept on a 13:11 light:dark cycle with heat lamps over basking areas turned on for 7 hours from 1000–1700 hours to facilitate behavioral thermoregulation, in which hatchlings appeared to engage extensively (Chapter 2). Five days per week, 150-W neodymium basking bulbs provided operative temperatures of $\geq 40^{\circ}\text{C}$ at the basking end of enclosures. Two days per week, 160-W Powersun UV mercury vapor bulbs (Zoo Med Laboratories, Inc., San Luis Obispo, California) provided lower operative temperatures and UVB radiation at the basking areas. When basking lamps were off, room air temperature remained between 22–26 °C. Room air temperature rose when basking bulbs were on, and depending on location within the laboratory, temperatures at the cool end of burrows ranged between 24–30 °C.

In July and August 2014, hatchlings were kept in incubators at 1 or 2 constant temperatures, ranging between 24–36 °C. Thirty individuals were released immediately thereafter on 1 September 2014. Twenty-eight individuals were maintained for an additional 22 days in a separate field laboratory near the release site. Temperatures ranged between 22–30 °C in this setting and individuals were taken outside periodically to forage on local grasses and forbs.

Radio transmitters and iButtons

I fitted 30 individuals with miniature radio transmitters and iButton temperature data loggers (Maxim Integrated Products, Sunnyvale, California, USA) that were programmed to record temperature at 10- or 30-min intervals. Prior to attachment, I coated transmitters and iButtons in brown rubber (Plasti Dip International, Blaine, Minnesota, USA) to provide waterproofing and camouflage. I used epoxy to attach transmitters to the posterior-most left or right costal scute and iButtons to the posterior-most vertebral scute. To avoid adversely affecting shell growth, I did not attach equipment to, or apply epoxy across, multiple scutes. Equipment and epoxy weighed approximately 5 g, or 5–9% of tortoise mass.

Hard releases

During the afternoon of 1 September 2014, I released 30 radio-transmitted yearling tortoises at or near their nest sites. If the nest site was at an active adult tortoise burrow, I released individuals several meters away to limit potential interactions with large individuals. I also found numerous ants at the nest site of one yearling and released that individual near another yearling's nest site located nearby. On 22 September 2014, after observing extensive burrowing and acceptable survivorship among individuals released three weeks earlier, I released 28 additional yearlings at or near nest sites without radio transmitters or iButtons. I measured midline plastron length (MPL) and mass for all tortoises prior to release (mean MPL = 63.3 ± 3.5 [1SD] mm, median = 63.7 mm, range = 55.9–69.1 mm). I repeated measurements during subsequent encounters of released tortoises through May 2016.

Radio telemetry

I tracked tortoises released with radio transmitters every 2–6 days for one month. When I located a tortoise, I recorded whether it was in a burrow, on a burrow apron (bare patch of soil just in front of the burrow), or at the surface but not on a burrow apron. For burrows constructed by radio-tracked tortoises, I measured distance to release location using a measuring tape.

I investigated whether tortoises released with radio transmitters dug burrow entrances under deadwood (sticks, logs, or bark > 2 cm in diameter) more often than would be expected by chance by determining presence or absence of deadwood at burrow locations and at random points. I located three random points at each burrow by extending a measuring tape a random compass direction and distance, ranging between 1–11 m, from each burrow. I considered random points to coincide with deadwood if deadwood was present within 10 cm. In one instance a random point corresponded to an adult tortoise burrow apron, and in another, a dirt road. I generated new random points in both instances because these were locations where juveniles are unlikely to burrow.

Antipredator behavior

I assessed responses of individuals on burrow aprons or in burrow entrances to simulated predator approach. Between 10 September and 10 October 2014, I performed simulated predator approaches on 23 of 24 yearlings released with transmitters on 1 September 2014 and known to be using a deep burrow. Wild juvenile gopher tortoises exhibit long flight initiation distances (FIDs), making it difficult to visually observe basking individuals and their responses to simulated predator approach directly (Radzio and O'Connor 2017; Chapter 3). Therefore, I used time-lapse video cameras (Plotwatcher

Pro, Day 6 Outdoors, Columbus, GA) to document tortoise responses. Prior to approach days, I mounted cameras on wooden poles next to burrows, trained cameras on burrow entrances, aprons, and nearby surrounding areas, and programmed them to continuously record (0.33 frames per second) during daylight hours.

I generally approached burrows by walking (~ 1.5 m/s) a straight path, typically starting ≥ 60 m away. For logistical reasons, in a small number of instances, I approached one burrow, then immediately approached a second burrow located nearby, thus, starting the second approach from a shorter distance. These short approaches were not used to estimate FID, but were used to determine if individuals hid and for how long. When I arrived at a burrow, I briefly placed my hand in front of the camera to record the exact time, information that was used to estimate FID (described below; Radzio and O'Connor 2017; Chapter 3). I then left the area, allowing the tortoise to emerge undisturbed. Burrows were approached only once per day, but this protocol was usually repeated on multiple days at the same burrow such that each tortoise was approached at least once as it basked on its burrow apron. Many basking individuals were approached more than once.

Activity, microhabitat use, and temperature relations

I used time-lapse video cameras, operative temperature models, iButtons affixed to tortoise shells, and iButtons placed inside the burrows of juveniles to quantify activity, microhabitat use, and temperature relations of released tortoises. Cameras recorded the activity of 19 yearlings at their burrows, each for one complete daylight period during 30 September–3 October 2014. To avoid influencing tortoise activity, I did not visit burrows when video recordings were made. A metal model (as described in Radzio and O'Connor

2017; Chapter 3), deployed in advance of video observations, recorded operative temperatures on burrow aprons at 10-min intervals. Similarly, iButtons attached to flexible tubing recorded temperatures at 10-min intervals and at different depths inside four small juvenile tortoise burrows located elsewhere on the study site.

Survivorship

I assessed short-term survivorship by radio tracking individuals fitted with radio transmitters ($n = 30$) during the month following their release (1–30 September 2014). The miniature radio transmitters I used had short battery lives, and in October 2014 I started removing them from individuals encountered away from burrows. Tortoises generally did not change burrows between fall and spring, enabling me to relocate most individuals visually through April 2015. To assess long-term apparent survivorship, I visually searched for tortoises released with ($n = 30$) and without ($n = 28$) radio transmitters through August 2016.

Data Analysis

I summarized distances between release sites and burrows initiated by tortoises fitted with radio transmitters. I also assessed the relationship between days since release and cumulative number of tortoises to have initiated a burrow. I tested for associations between burrow locations and deadwood by analyzing observed and expected frequencies of burrows and random points with and without deadwood using a chi-square goodness-of-fit test.

I compared the FIDs of released yearlings to an FID estimate for 12-month-old wild individuals (31.5 m; Radzio and O'Connor 2017; Chapter 3) using a one-sample t-test. I used a Mann-Whitney U test to compare hiding times of released yearlings to those

of 0.5–4.0 year-old wild tortoises reported in Radzio and O'Connor (2017; Chapter 3). I defined hiding time as time until any portion of the disturbed tortoise reappeared outside the burrow. When multiple FID or hiding time observations were obtained for the same individual, I used mean values in statistical comparisons.

I qualitatively compared activity, microhabitat use, and thermal relations of released tortoises to literature accounts and my observations of wild juvenile gopher tortoises. Wild juveniles use burrows at night and spend limited time away from the relative safety of burrows or burrow aprons during the day (Wilson et al. 1994; Pike and Grosse 2006; Radzio and O'Connor 2017; Chapters 2–3). Observations of wild juveniles at the study site indicated that surface activity was dominated by basking in burrow entrances or on burrow aprons. Although the extent to which juveniles bask is temperature-dependent, they routinely engage in this activity for multiple hours each day (Chapter 2).

I quantified growth (Δ MPL, mass) during the first 1–2 months following release ($n = 18$ individuals released on 1 September 2014) and during the first 19–20 months following release ($n = 11$ individuals released on 1 and 22 September 2014). I compared post-release growth (Δ MPL) during the first 19–20 months following release to the expected growth of wild individuals at the study site during the same time intervals using a Wilcoxon matched-pair signed-rank test. I derived expected growth rates for each time interval as the product of the annual MPL growth rate of wild tortoises (age 0–4 years) at the site (13.5 mm per year; Radzio, unpublished data) and the number of growing years represented by the interval, with the latter calculated to reflect seasonal growth of tortoises in southwest Georgia (using Landers et al. 1982).

Results

Burrowing behavior

At least 14 of 30 (47%) radio-tracked tortoises initiated burrow construction within 2 days of hard release; ≥ 22 (73%) initiated construction within four days; and ≥ 27 (90%) had initiated a burrow within 13 days (Figure 1). I documented a 28th tortoise constructing a burrow 21 days after release. The two tortoises that did not appear to initiate burrows were found dead, apparently depredated, four and six days following release. In total, I documented 36 burrowing attempts by 28 radio-transmitted yearlings during their first month following hard release. On average, tortoises constructed burrows 29.4 ± 29.9 m (median = 20.6 m, range = 3.0–135.2 m) from release sites, and 31 of 36 (86%) burrows were located < 50 m from release sites. Tortoises observed outside burrows were always found near release sites or their burrows. Depredated tortoises were also located near (< 200 m) release sites.

Burrows constructed by tortoises released with radio transmitters were positively associated with deadwood ($\chi^2 = 19.8$, $p < 0.00001$). Fifteen of 36 (42%) burrow entrances were constructed under deadwood, while only 10 of 108 (9%) random points fell within 10 cm of deadwood. Additionally, three transmitted tortoises constructed burrows in stumpholes (not classified as deadwood in my analyses).

I relocated 11 of 58 (19%) tortoises in early August 2016, nearly two years following hard release. These individuals were using burrows located approximately 29.9 ± 36.7 m from release sites (median = 16.6 m, range = 3.0–135.2 m). Distances are close approximations because measurements were made from nest sites. Some yearlings

were released several meters away from nest sites, and exact release locations were not recorded.

Apparent burrow defense behavior

On 2 September 2014, I radio-tracked a yearling that had been hard-released the previous day to its new burrow. I placed a coaxial cable attached to a radio telemetry receiver inside the burrow to confirm presence of the yearling. The yearling responded by repeatedly ramming the coaxial cable with its gular and continued to do so as the cable was slowly retracted to the surface. Later, in early October 2014, I relocated this yearling at a small juvenile tortoise burrow. I wanted to capture the tortoise to remove its iButton (its transmitter had detached prematurely due to epoxy failure) and obtain growth measurements. The juvenile vigorously rammed the dowel with its gular protrusion and continued to do so as the dowel was slowly pulled out of the burrow. In July 2016, I successfully used the same technique to capture another tortoise hard-released nearly two years earlier. In all three instances, the juveniles appeared highly motivated to exclude the coaxial cable or dowel rod from their burrow, mirroring aggressive responses previously noted in wild hatchling and juvenile gopher tortoises at this site (Radzio et al. 2016).

Activity, microhabitat use, and temperature relations

Tortoises emerged from burrows between 0730–1000 h (Figure 2). Basking at burrow entrances or on burrow aprons accounted for most surface activity. On average tortoises spent 7.6 ± 1.3 h (median = 7.3 h, range = 4.8–10.0 h) at the surface, of which 6.4 ± 1.7 h (median = 6.2 h, range = 2.0–9.8 h) was spent in burrow entrances or on burrow aprons, and 1.2 ± 1.8 h (median = 0.8 h, range = 0.0–8.0 h) was spent away from

burrows. One yearling left its burrow area around 1030 h, and did not return until the following day. The iButton temperature profile for this individual indicated that it spent the night above ground or in a shallow burrow. All other tortoises returned to their burrows by 1540 h. By dusk (when cameras turned off) tortoises were fully inside their burrows, but four were still inside burrow entrances, with a small portion of their bodies outside (Figure 2). One yearling was not video-recorded because it was apparently not using a burrow for several days (see “Survivorship” subsection for additional details).

Deep (50–150 cm) burrow temperatures remained constant at 25 °C throughout the diel cycle. Burrow temperatures near (10 cm) the surface fluctuated more. Surface operative temperatures typically exceeded burrow temperatures from midmorning to late afternoon. By engaging in surface activity, tortoises could elevate shell temperatures to within preferred body temperature ranges during much of the day on warm days, but not on cool days (Figure 3). Tortoise basking behavior and shell temperatures relative to available surface operative temperatures indicate tortoises took advantage of most opportunities available on burrow aprons to maintain elevated temperatures (Figure 3).

Antipredator responses

I observed a small number of instances where individuals siting on burrow aprons did not attempt to hide in burrows when I arrived. All but one such instance of atypical behavior occurred within 9 days of release.

Tortoises on burrow aprons always hid inside burrows in response to simulated predator approaches (n = 41 approaches of 23 basking tortoises), which were initiated 9 days after release. This included two tortoises that were facing into burrows with their heads fully belowground when approached. In most instances, flight movements into

burrows were rapid, and if tortoises were facing away from burrow entrances, they generally backed into the burrow, rather than first turning around to face the entrance before entering. Mean FID was 34.1 ± 14.5 m (median = 34.5 m, range = 2.3–58.5 m, $n = 20$) and did not differ from the predicted FID of wild 12-month-old gopher tortoises (31.5 m; Radzio and O'Connor 2017, Chapter 3; $t_{19} = 0.791$, $p = 0.439$). When all observations were used, hiding times of released tortoises (mean = 34.9 ± 76.7 min; median = 6.8 min, range = 1.8–360.0+ min) did not differ from those of small wild juvenile tortoises reported in Radzio and O'Connor (2017, Chapter 3; $U_{41} = 162.0$, $p = 0.098$). However, when we excluded two observations where released yearlings did not emerge from burrows before cameras stopped recording (approximately 2 and 6 h later), hiding times of released individuals were shorter (mean = 15.4 ± 20.6 min; median = 5.8 min, range = 1.8–69.5 min) than those of wild individuals ($U_{39} = 298.0$, $p = 0.022$).

Released yearlings also engaged in other antipredator behaviors exhibited by wild gopher tortoises at this site. For example, individuals encountered away from burrows would often become motionless. Additionally, I attempted to remove a yearling from a shallow burrow on one occasion, but it wedged itself very securely inside by lifting up with its forelimbs such that its carapace pushed firmly against the top of the burrow shaft.

Growth

Tortoises released with radio transmitters on 1 September 2014 exhibited little change in MPL (0.3 ± 0.7 mm, median = 0.2 mm, range = -0.9–1.4 mm, $n = 18$) or mass (-0.1 ± 3.1 g, median = 0.0 g, range = -4.3–4.9 g, $n = 18$) when recaptured 1–2 months later. Similarly, two other individuals also released on 1 September but not recaptured in the fall, exhibited little change in MPL (-0.25 and 0.12 mm) or mass (-8.9 and -8.6 g) when

recaptured in early April 2015. However, 11 tortoises, comprising 6 individuals released on 1 September 2014 and 5 released on 22 September 2014, exhibited substantial increases in MPL (17.1 ± 3.0 mm, median = 18.5 mm, range = 12.1–21.2 mm) and mass (55.3 ± 17.6 g, median = 60.7 g, range = 23.6–79.5 g) when recaptured approximately 19–20 months later in early May 2016. Change in MPL of released tortoises did not differ from change in MPL predicted for wild tortoises at this study site during the same time intervals (mean = 17.5 mm, $W = 29.0$, $n = 11$, $p = 0.722$).

Survivorship

Nineteen to 23 of 30 (63–77%) yearlings hard-released with radio transmitters on 1 September 2014 survived through at least 1 April 2015 (Figure 4). Three tortoises were depredated by cottonmouths (*Agkistrodon piscivorus*), and one was taken by an unidentified mammal. Two others were apparently depredated by red imported fire ants (*Solenopsis invicta*). A seventh individual was not found, but was likely depredated as its transmitter, discovered in a nearby lowland area, had bite marks on it. Four others could not be relocated. At least 11 of 58 (19%) animals released with or without transmitters in September 2014 survived through 1 August 2016.

I may have artificially increased survival odds for one radio-transmitted yearling. On 1 October 2014, I located the yearling about 20 m away from its burrow and collected it to remove its radio transmitter and iButton and to measure growth. The tortoise's burrow appeared inactive, and its iButton thermal profile indicated that it had spent the previous 1–3 nights above ground, an unnatural behavior. Instead of returning it to where I found it, I placed it inside its burrow, which it continued using through at least 1 August 2016.

Discussion

Despite having spent their first year of life in captivity, yearling gopher tortoises exhibited many traits required to perform well in the wild, including burrowing and antipredator behaviors (Douglass 1978; Wilson et al. 1994; Radzio and O'Connor 2017). Within days of release, most laboratory-reared tortoises initiated burrows near their release sites. Similar to wild juvenile gopher tortoises, released individuals disproportionately placed burrow entrances under deadwood (Aresco 1999) and spent most of their time in or near these refugia (Wilson et al. 1994; Pike and Grosse 2006). Video observations indicated released yearlings engaged in extensive surface activity, which was dominated by basking on burrow aprons, as documented for wild individuals at this site (Radzio and O'Connor 2017). By basking on burrow aprons, released yearlings increased body temperatures over those achievable in burrows and maintained shell temperatures near the preferred body temperatures of fed hatchlings ($\sim 32\text{--}33^\circ\text{C}$; Johnston 1996; Radzio and O'Connor, unpublished data), especially on warm days. Strong thermal constraints on digestion in herbivorous ectotherms (Troyer 1987; Zimmerman and Tracy 1989; van Marken Lichtenbelt 1992; Tracy et al. 2005) suggest basking increases growth rates of young gopher tortoises.

Wild hatchlings and juveniles manage predation risk associated with thermoregulation by limiting basking to burrow aprons and entrances and quickly hiding below ground in response to potential predators (Wilson et al. 1994; Radzio and O'Connor 2017). Remarkably, despite having been raised in an artificial environment and interacting daily with humans for the first year of their life, released tortoises always hid in burrows during simulated predator approaches and exhibited flight initiation distances

that were similar to those of wild individuals (Radzio and O'Connor 2017). Captive-reared tortoises usually hid before they could be observed and appeared to use vibrations (aerial or ground) to detect the simulated predator just as wild hatchlings and juveniles do (Radzio and O'Connor 2017; Chapter 3). When I excluded two outlier observations, hiding times of released tortoises were slightly shorter than those of wild hatchlings and juveniles (Radzio and O'Connor 2017; Chapter 3). One reason for this difference could be that burrow temperatures were generally cooler when tortoises were approached in this study. Nevertheless, both wild and released individuals reemerge soon after hiding, indicating a strong inclination to bask (Radzio and O'Connor 2017; Chapters 2–3) and potential physiological benefits associated with maintaining high body temperatures (Chapter 2).

In addition to increased predation risk, translocation can sometimes induce physiological stress and related problems (Cook 1983; Field et al. 2007; Teixeira et al. 2007). Although operative temperatures in direct sunlight regularly exceeded lethal limits during the day following the first hard release (Radzio and O'Connor, unpublished data), I documented no physiologically-related mortality events, suggesting yearlings avoided overheating by using shaded or subsurface microhabitats. Hathaway (2012) reported lower fecundity in recently relocated female gopher tortoises relative to resident females and attributed this difference to translocation stress. I observed negligible growth during the first 1–2 months following release for animals fitted with transmitters. Wild juvenile gopher tortoises at this latitude grow little in September–October (Landers et al. 1982), but low growth during this period could also have reflected relocation stress or other factors. Consistent with the normal behaviors of the released study animals after having

established a burrow, growth of individuals recaptured more than one year later was indistinguishable from that of wild juveniles at the site.

Selective foraging behavior likely plays an important role in shaping juvenile gopher tortoise growth rates, particularly given preferential consumption of plants high in nitrogen (Mushinsky et al. 2003). Although I did not evaluate the diets of released yearlings, lack of detectable difference in growth rate between released and wild individuals suggested lab-reared tortoises foraged appropriately. However, I did document that, like wild juveniles, released yearlings generally limited movements away from burrows to short intervals during midday hours, when many tortoise predators are presumably less active (Johnston 1996; Halstead et al. 2007; Radzio 2017).

My data do not permit rigorous comparison of survivorship following hard release versus soft release. However, based on available literature values from other sites, survival nearly two years following hard release appeared consistent with what might be expected for soft-release. Tuberville et al. (2015) report apparent survivorship of 0.35 during the year following soft-release for gopher tortoises released at about 9 month of age, and Wilson (1991) reports annual survivorship of 0.45 for small juveniles (age = 1–4 year). Using these values, one might expect to relocate about 9 of our 58 released yearlings 2 years later ($[58 \text{ released animals} \times 0.35 \text{ first-year survivorship}] \times 0.45 \text{ second-year survivorship} = 9.1$). Correspondingly, I relocated 11 of 58 (19%) individuals, nearly 2 years following hard-release.

Naturally low growth rates and survivorship of small juveniles combine to limit the effectiveness of turtle captive breeding and headstarting programs (Frazer 1992; Heppell et al. 1996). Nonetheless, such measures may represent critical lifelines for some

critically endangered species with very low numbers in the wild (Gibbs et al. 2014; Spencer et al. 2017). Establishing viable turtle populations through release programs can require long-term commitment and effort (Gibbs et al. 2014), but funding for conservation activities is often limited, short-term, and highly variable (Snyder et al. 1996). Recent work with Hermann's tortoises (*Testudo hermanni*; Lepeigneul et al. 2014) and gopher tortoises in this study suggests hard release can provide an alternative to soft release in some situations. Availability of hard release as an option can make captive breeding and headstarting programs easier and less expensive to implement, which should promote continuity and increase success.

This study indicates hard release represents a responsible method for releasing laboratory-reared gopher tortoises, but my observations also suggest a shortcoming of this approach. In this study, 5–7 of 30 (17–23%) radio-tagged yearlings were depredated before having constructed a long burrow. It is possible that I could have reduced initial mortality by releasing yearlings in areas with fewer roots, providing them with starter burrows, or protecting individuals against predators until they constructed burrows (Tuberville et al. 2015; Quinn 2016). However, juvenile gopher tortoise soft-releases also report substantial initial mortality, and penning can concentrate and confine individuals to small areas or along fence lines which can render them more vulnerable to depredation by fire ants, dogs, and, potentially, other animals (Holbrook et al. 2015; Tuberville et al. 2015; Quinn 2016).

Behavior, growth, and survival documented here for hard-released yearling gopher tortoises suggests captive-reared juvenile tortoises can integrate into wild populations. However, my data also indicate that, similar to small wild juveniles, few

released yearlings survive to maturity (Wilson 1991; Frazer 1992; Heppell et al. 1996; Tuberville et al. 2015). Releasing tortoises when they are older and have achieved larger body sizes might reduce mortality during the vital period when individuals have yet to adopt or construct a burrow. Indeed, Tuberville et al. (2008) documented high survival of large, translocated juvenile and adult gopher tortoises and the establishment of a viable population. However, the role that captive breeding or headstarting should play in turtle conservation requires careful consideration, particularly with respect to available options (Frazer 1992; Heppell et al. 1996; Spencer et al. 2017) and the potential for disease spread and introduction. Nevertheless, my findings indicate that some turtles are behaviorally resilient enough such that hatchlings can be studied in the laboratory and subsequently hard released successfully. Furthermore, studies based on released turtles as study subjects can provide accurate information relevant to the biology and conservation of these animals.

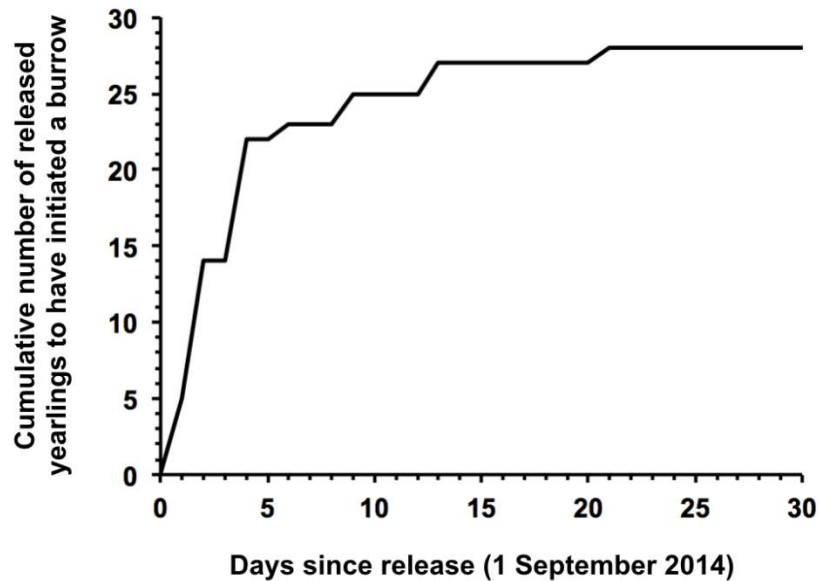


Figure 0-1. Cumulative number of laboratory-reared yearling gopher tortoises that initiated a burrow in relation to days since hard release. Tortoises were released on 1 September 2014 near their nest sites and monitored using radio transmitters ($n = 30$). Two tortoises that did not initiate burrows were found dead on 5 and 7 September 2014. One was destroyed, apparently by a mammal, and the other appeared to have succumbed to red imported fire ants. The study area was mature, longleaf pine forest in southwest Georgia, USA.



Figure 0-2. Diurnal microhabitat use patterns of 19 yearling gopher tortoises approximately 1 month following hard release at or near their nest sites in a longleaf pine forest in southwest Georgia, USA. Each horizontal bar depicts microhabitat use for one individual video-recorded on one diurnal period between 30 September and 3 October 2014. Microhabitat use was point-sampled from video observations at 10-min intervals and extrapolated.

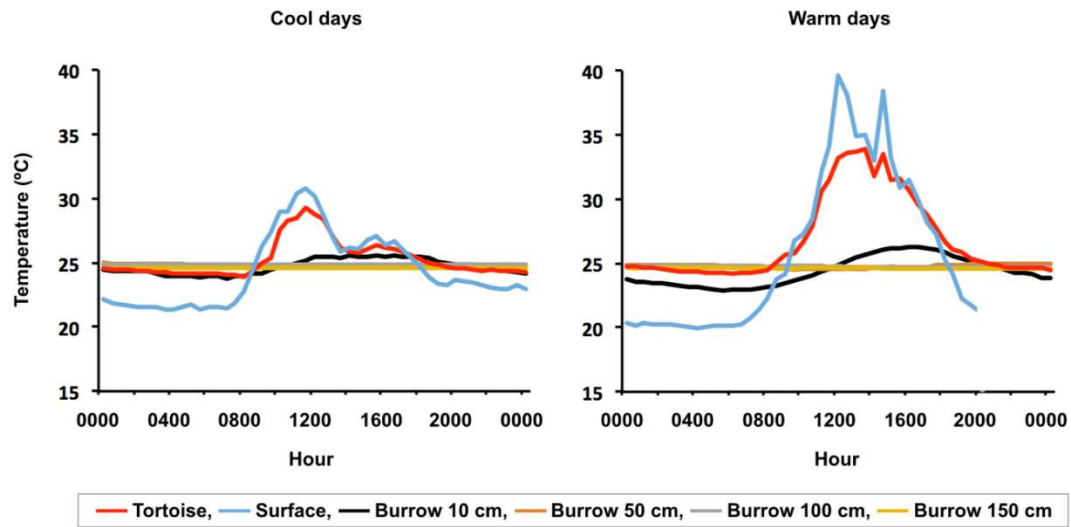


Figure 0-3. Mean shell and environmental temperatures for 16 yearling gopher tortoises on warm and cool days approximately 1 month following hard release at or near their nest sites in a longleaf pine forest in southwest Georgia, USA. Data are for nine yearlings, observed on one of two cool days, and seven other yearlings, observed on one of two warm days, occurring between 30 September and 3 October 2014.

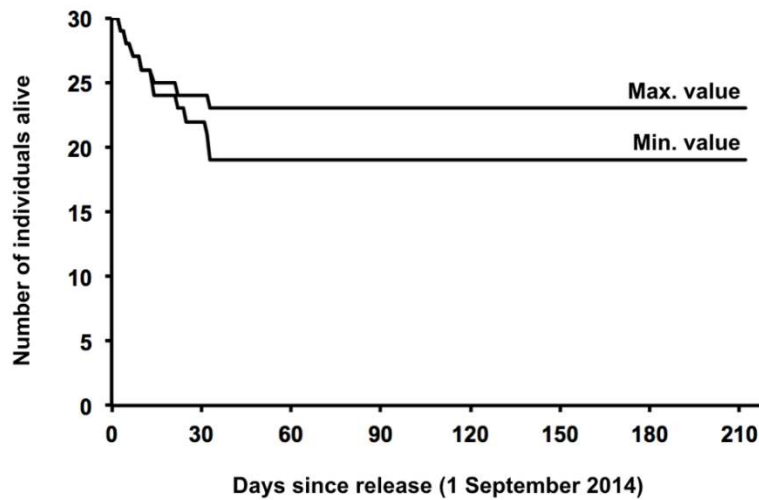


Figure 0-4. Short-term survivorship of 30 laboratory-reared yearling gopher tortoises hard-released with radio transmitters at or near their nest sites on 1 September 2014. Tortoises were radio-tracked for one month before I began removing transmitters in October 2014. Marked individuals continued using established burrows through the fall and winter, enabling resighting of most yearlings for several months. Minimum and maximum survivorship values reflect failure to relocate individuals, including tortoises whose transmitters detached prematurely from shells, apparently due to epoxy failure. I also investigated survivorship over a longer period by visually searching for individuals released with or without radio transmitters in September 2014. As of August 2016, 11 of 58 (19%) juveniles were known to be alive.

CHAPTER 7: CONCLUSIONS

Many ectotherms regulate body temperature behaviorally, which can enhance physiological processes such as digestion and growth, but thermoregulatory activities, including basking and shuttling movements between sun and shade, can expose individuals to increased predation risk (Lillywhite et al. 1973, Huey 1974, Huey and Slatkin 1976, Angilletta 2001). These and other costs may influence the extent to which reptiles thermoregulate and can impose constraints on physiological performance and life history traits (Huey 1974, Huey and Slatkin 1976, Vickers et al. 2011, Sears et al. 2016). However, limited basking may not be an optimal, or even demographically feasible, strategy if juveniles grow very slowly and remain vulnerable to predators during other activities. Strong thermal constraints on digestion in herbivores (Troyer 1984, Bjorndal 1987, Zimmerman and Tracy 1989) might favor extensive thermoregulation by young gopher tortoises, particularly in cool environments.

In Chapter 2, I investigated thermoregulatory strategies and thermal constraints on growth in neonate, hatchling, and small juvenile gopher tortoises. Findings indicate hatchlings and small juveniles exploit most thermal opportunities available at burrows during daytime hours, basking increases growth rates, but low environmental temperatures likely constrain growth throughout the active season in a Georgia longleaf pine forest. Extensive basking begins at the neonate stage, where young exhibit a grow-fast strategy that exposes them to predation risk during thermoregulatory activity, but is presumably optimal overall. Although young bask extensively, their body temperatures may be particularly influenced by shade because they limit basking to just in front of burrows (Wilson et al. 1994, Johnston 1996). The field and laboratory data also imply

that tortoise temperatures and thermosensitivity of growth are such that small changes in environmental temperature, including those that could be incurred by increased shading (Aresco and Guyer 1999a) or climate change (IPCC 2014) can substantially impact growth rates and, therefore, time spent at small body sizes, where individuals are most vulnerable to predators.

In Chapter 3, I examined how behavior and thermal environment influence a trade-off between thermoregulation and predator avoidance in hatchling and juvenile gopher tortoises. Basking tortoises always hid inside burrows when approached and exhibited very long flight initiation distances that increased with age/size. Individuals fled into burrows even when it was not possible for them to see the approaching researcher, suggesting use of vibrations (aerial/ground) to detect potential predators and a possible antipredator function for exceptionally large otoliths characteristic of the species (Bramble 1982, Bramble and Hutchison 2014). Tortoises hid for short durations, especially in cool burrows, suggesting that hiding responses are sensitive to physiological costs of refuge use. Additionally, surface activity following disturbance consisted primarily of basking and correlated negatively with burrow temperature. These findings suggest that thermal environment influences predation risk in ectotherms whose surface activity is driven primarily by thermoregulatory requirements and highlight potential benefits of warm, well-insolated habitats, such as endangered longleaf pine ecosystems, for juvenile gopher tortoises.

In Chapter 4, I examined thermosensitivity of hatchling righting response in the laboratory and combined findings with field observations to assess overheating risk associated with overturning during activity. In the field, hatchlings experienced high shell

temperatures during activity away from burrows and often moved through sunlit areas where operative temperatures could be lethal for immobilized individuals. Hatchlings overturned when negotiating uneven terrain, such as small logs, and during aggressive conspecific interactions (Radzio et al. 2016; Chapter 5). Field hatchlings always initiated righting attempts within seconds of overturning and, though they sometimes required multiple attempts, always righted faster than would be required to avoid overheating in the hottest conditions encountered during activity. In the laboratory, latency times on both tested substrates and active righting times on sand augmented with wire mesh to enhance grip generally decreased with increasing temperature. Active righting times on sand augmented with wire mesh, especially in high temperature treatments, were usually short enough to avoid overheating at any temperature encountered by wild individuals. However, hatchlings were essentially unable to right fast enough on loose sand without wire mesh to avoid overheating under hot field conditions, suggesting that overturning carries some small, but finite risk. Short latency and active righting times appear well suited for hatchling gopher tortoises, small ectotherms that forage in hot environments, climb over obstacles, and engage in combat with conspecifics.

Chapter 5 describes social interactions relevant to the thermal ecology of immature gopher tortoises. Immature tortoises usually inhabited burrows alone but occasionally shared them. Presence of multiple individuals inside burrows hindered putative thermoregulatory shuttling movements. Hatchlings and juveniles exhibited overt aggression toward similar age/size conspecifics in about one-half of encounters, and cameras may have failed to detect aggression occurring inside burrows. Hatchlings and juveniles also exhibited aggressive responses to foreign objects that were similar

in appearance to small tortoises. Aggressive behaviors resembled those of adults and included repeated ramming, pushing, biting, and flipping. Young tortoises may respond aggressively to conspecifics at burrows because the presence of additional individuals constrains thermoregulatory and antipredator movements within these important microhabitats. Future studies could examine potential costs associated with burrow cohabitation (e.g., constraints on thermoregulation and predator avoidance), individual variation in aggressive tendency, factors that may underlie such potential variation (e.g., personality, age, sex), and how aggressive interactions influence patterns of burrow use and cohabitation in young gopher tortoises.

I implemented work in Chapter 6 in responses to concerns raised by the Georgia Department of Natural Resources that the hatchling tortoises raised in captivity for thermal physiology experiments may require special release measures to perform well after being returned to the study site. Maintaining animals in captivity constitutes a vital component of many conservation and research programs (Swimmer et al. 2005, Crone et al. 2007, Griffiths and Pavajeau 2008). However, releasing captive animals into the wild can present challenges because released individuals often experience high predator-induced mortality (Moseby et al. 2011, Holbrook et al. 2015, Tuberville et al. 2015). Released individuals can be more vulnerable to predators than wild counterparts for various reasons, including because they are unfamiliar with their surroundings, have yet to establish refugia, and, in some cases, exhibit unnatural behaviors (Beck et al. 1994, Tuberville et al. 2015).

I hard-released laboratory animals after they spent nearly the first year of their life in captivity. Individuals were released with radio transmitters, such that if major

problems arose, I could collect them and implement soft release measures. If hard release worked well, it could be a useful option for future studies. Most yearlings fitted with radio transmitters constructed burrows soon after release, and, like wild juveniles, burrowed under deadwood more frequently than would be expected by chance. Subsequent video observations indicated normal activity and microhabitat use, including overnighing in burrows, extensive basking on burrow aprons, and limited time spent away from burrow areas. Growth rate also did not differ from that of wild juveniles at the site. Despite daily contact with researchers when in captivity, released tortoises always hid in burrows during systematic approaches and exhibited long flight initiation distances and short hiding times comparable to those of wild individuals. Hard-released tortoises also displayed survivorship consistent with what would be expected following soft release, and all known mortalities were apparently due to predators, particularly cottonmouths (*Agkistrodon piscivorus*). These findings indicate laboratory-reared gopher tortoises can be responsibly hard-released back into nature, retain critical attributes required to perform well in the wild, and can be studied following release to obtain accurate information useful for conservation and ecological research activities.

This investigation raises additional questions associated with the thermal ecology of young gopher tortoises. For example, future work might examine potential roles of thermal environment in burrow site selection and abandonment (Guyer and Hermann 1997, Aresco and Guyer 1999b), thermoregulatory activity within burrows at night (Johnston 1996) and its energetic consequences, temperature effects on basking activity and interactions with predators (Radzio and O'Connor 2017; Chapter 3), and potential climate change impacts on tortoise vital rates and population persistence. This study also

highlights challenges and opportunities associated with conservation and management of northern gopher tortoise populations, where thermal constraints on growth may contribute to low juvenile recruitment and aging populations (Epperson and Heise 2003, USFWS et al. 2013).

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Appendix A: Behavior and Conspecific Interactions of Nesting Gopher Tortoises (*Gopherus polyphemus*)

Abstract

Nesting behavior, including nest site selection, has important consequences for many egg-laying reptiles because it can influence egg depredation rates, embryonic development, and offspring characteristics. I investigated nesting behavior in a population of Gopher Tortoises (*Gopherus polyphemus*) inhabiting an old-growth Longleaf Pine (*Pinus palustris*) forest in southwest Georgia (USA) using time-lapse video cameras set to record nesting activity in front of tortoise burrows. Information on nesting in this species is primarily limited to frequency, seasonal timing, and location; actual nesting behavior remains incompletely described for wild individuals. Females engaged in nesting activity at their own burrows and at other burrows during late May to mid June from 1000 to about 1800 h. Tortoises exhibited wide variation in nest site preparation activity, ranging from no preparation to circling and constructing a shallow depression. Nesting females faced away from burrows, braced with forelimbs, and used hindlimbs to dig nest cavities, arrange oviposited eggs, and initially cover nests. On average females spent 74 min constructing nests, but about twice as long manicuring nest sites thereafter. Manicuring females repeatedly nuzzled the ground, kicked dirt out of burrows onto nests and surrounding areas, and roughed up the soil, perhaps to assess and obscure olfactory and visual cues available to potential nest predators. Notably, on multiple occasions females abandoned nesting attempts in response to conspecifics. Additional observations of conspecific interactions at burrows, particularly aggressive

mating attempts and female-female combat involving gravid individuals, further indicate that tortoises routinely interact in ways that can interfere with nesting and influence where individuals nest.

Introduction

Nesting behavior can influence reproductive success and offspring quality in oviparous species. For example, nest-site selection by female turtles can determine incubation environment and affect embryonic development, hatching success, and offspring performance (Wilson 1998, Booth et al. 2013). In many turtles, nest temperature also determines offspring sex, which may be important when demographic or environmental characteristics benefit one sex over the other (Charnov and Bull 1977, Bull and Vogt 1979). In addition to carefully selecting nest sites, some turtles camouflage nests from predators or actively guard nests (Hailman and Elowson 1992, Agha et al. 2013 and references therein). External factors such as nest-site availability or human disturbance may influence nesting behavior (Roosenburg 1991, Johnson et al. 1996), but conspecific interactions are not thought to interfere with nesting activities in most turtles (but see Hughes and Richard 1974, Doody et al. 2009).

Gopher Tortoises (*Gopherus polyphemus*) inhabit dry upland areas along the Coastal Plain of the southeastern United States. Individuals center daily activities on long (often > 4 m) burrows that they excavate and use for thermoregulation, predator avoidance, rest, and other activities (Douglass and Layne 1978). In part because tortoises spend most of their time at burrows, these sites also serve as primary locations for mating

and competitive intrasexual interactions (McRae et al. 1981, Douglass 1986, Diemer 1992, Johnson et al. 2007, Guyer et al. 2014). Additionally, in many populations, females often, perhaps predominantly, nest just in front of burrows in the mound of bare soil referred to as the burrow apron (Landers et al. 1980, Butler and Hull 1996, Epperson and Heise 2003, Hammond 2009, Lamb et al. 2013). Gopher Tortoises potentially prefer relatively sunlit burrow aprons or other warm bare ground areas as nest sites, but such sites may be less common in areas where fire suppression has allowed dense hardwood shrubs and small trees to dominate (Diemer 1986, Diemer and Moore 1993, Averill-Murray et al. 2014).

I investigated the nesting behavior of Gopher Tortoises in an old-growth forest dominated by an overstory of Longleaf Pine (*Pinus palustris*) and a diverse groundcover community composed of hundreds of native forbs and grasses. Although Gopher Tortoises have been studied extensively, aspects of their nesting behavior beyond frequency, seasonal timing, and location (e.g., cavity construction, egg laying) remain largely undescribed. Gopher Tortoise behaviors can be difficult to document due to the tendency of individuals from many populations to quickly hide in burrows when approached (Anderson 2001, Radzio and O'Connor 2017, Chapter 3). Butler and Hull (1996) briefly observed two wild female Gopher Tortoises digging nest cavities, but both tortoises abandoned nesting attempts before ovipositing, apparently in response to researcher presence. The most complete account of an actual nesting event is for a single captive individual kept far north of the range of the species in Connecticut, USA (Kenefick 1954).

I used time-lapse video cameras to document the natural, undisturbed behaviors of nesting Gopher Tortoises before, during, and after oviposition. My observations comprised nesting activity as well as interactions between tortoises, including gravid females, at nest sites. This work provides new insights into tortoise social interactions and how they might influence nesting activity in this secretive species.

Materials and Methods

Study site description

I studied Gopher Tortoise nesting and conspecific interactions on Wade Tract (30°45'N, 84°0'W), an 80-ha old-growth ecological preserve located near Thomasville in southwestern Georgia, USA. Longleaf Pines, many greater than 200 y old, dominated the upper canopy (Platt et al. 1988). Ground cover included relatively few bare spots and was dominated by Wiregrass (*Aristida stricta*), oak (*Quercus* spp.), and other native plants (Christine Ambrose, unpubl. report). Wade Tract is located within Arcadia Plantation, a 957-ha area that consists primarily of mature (> 80 y) Longleaf Pine forest. Wade Tract is managed by Tall Timbers Research Station using frequent prescribed fire (≤ 2 -y return intervals), and surrounding areas of Arcadia Plantation are burned at similar intervals. Previous work at Wade Tract reports a site-wide density of adult tortoises of 0.8 individuals/ha (Guyer et al. 2012). However, adult tortoise densities at locations within the site where I conducted observations were higher (T. Radzio, unpubl. data).

Observations of nesting and conspecific interactions

I set time-lapse video cameras (Plotwatcher Pro, Day 6 Outdoors, Inc., Columbus, Georgia, USA) to record activity at tortoise burrows known or thought to potentially contain adult female tortoises. I attached cameras to wooden stakes and positioned them to monitor burrow entrances, burrow aprons, and surrounding areas. I programmed video cameras to record a time-stamped frame every 5 seconds during daylight hours, except for a very small number of days when I set video cameras to record a frame every 1 second. Tortoises did not appear to respond to the presence of cameras.

During mid-May to late-June 2013, video cameras monitored the activities of 10 female tortoises at their burrows, hereafter referred to as resident tortoises 1–10 that could be individually identified by the presence of radio transmitters (used in another study) or unique shell markings. Resident females were monitored for 2–33 complete days (median = 20 days; complete day = video collected from at least 0600–1900 h, but usually dawn to dusk; all times reported in Eastern Standard Time). Additionally, cameras monitored activity at two other burrows, both occupied by adult males, for 5–11 complete days. I refer to tortoises that appeared at the burrows of resident tortoises as visitors.

From mid-May to mid-June, I visited tortoise burrows on most days to search for nests. In general, I spent little time at individual burrows, allowing tortoises to engage in natural, undisturbed activity and behavior throughout most of the day. I rarely dug into burrow aprons to locate nests, but instead relied on signs of potential nesting activity such as disturbance to burrow aprons and presence of fresh soil behind burrow entrances (Matt Hinderliter, pers. comm.). When I located a nest, I initially covered it with a small piece

of hardware cloth, buried several cm below the soil surface, to protect eggs against predators (Radzio et al. 2017). Only in one of six instances where females completed a nest on video did I dig in burrow aprons or install a nest protector during the remaining daylight period following nesting. In that instance, I partially excavated the nest and covered it at 1900 h, more than 3 h after the female completed nesting. Therefore, I only minimally influenced nest manicuring activity. In most cases, I allowed cameras to continue recording tortoise activity at nest sites for several days following nest discovery.

Data analysis

I viewed video recordings in GameFinder software (Day 6 Outdoors, Columbus, Georgia) and scored all nesting attempts, conspecific visits, and social interactions. I assessed whether females dug nest cavities and oviposited with their heads oriented non-randomly relative to burrow entrances (either facing at least partially toward or at least partially away from the burrow) using a binomial test. I also evaluated variation in the time that females spent in different stages of nesting (identified and described in Results) using a Kruskal-Wallis test. I used this nonparametric test because sample sizes were too small to assess assumptions of parametric procedures. I performed posthoc pairwise comparisons using Bonferroni-corrected Mann-Whitney U tests. I conducted statistical analyses in SPSS version 24.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 24.0.) with $\alpha = 0.05$.

I describe encounters between nesting tortoises and conspecifics. I also characterized encounters between females and conspecifics outside of obvious nesting activity, but during the nesting season. To characterize interactions between females, I

determined the proportion of female-female encounters (multiple individuals visible at the same time on video) that included at least one female responding aggressively to the other. I evaluated aggression at two levels. I considered an interaction to be aggressive if at least one female rammed the other. I considered the interaction potentially aggressive if one female blocked the burrow entrance. I used the same approach to describe encounters between females and large juveniles.

To characterize female-male interactions, I determined the proportion of female-male encounters in which the male attempted to mate with the female. I identified male mating behavior to include head bobbing or mounting attempts. I also calculated the proportion of female-male encounters and mounts that represented potentially successful mating attempts. Potentially successful matings appeared to be distinct from other mating attempts in that males remained mounted longer, made deep head thrusts, and dismounted females voluntarily. Males left female burrow areas immediately after potentially successful mating attempts. I did not assess aggression between females and males because subtle aggression is difficult to distinguish from mating activity.

Results

Summary of nesting observations

Cameras documented six complete and six abandoned nesting attempts on Wade Tract by six resident and three visiting females ($n = 9$), all commencing between 1009–1736 h on 25 May to 13 June 2013 (Table 1). Additionally, while conducting fieldwork, I observed a tenth female digging a nest cavity on a burrow apron outside of Wade Tract, but still on Arcadia Plantation in the late afternoon of 5 June 2013. She abandoned the

attempt before ovipositing, but a nest was laid in the same location 2 days later. I found no indication of tortoises laying eggs more than once during the nesting season. All observed nesting attempts occurred on burrow aprons, approximately even with to three adult tortoise body lengths from the back edges of burrow entrances.

Tortoises did not limit nesting activity to their own burrows. Three visiting females attempted to nest (three observations) or nested (one observation: after the resident female left) at burrows of video-recorded conspecifics (Table 1). Resident females also may have nested at other burrows. One resident female left her burrow at 1011 h on 9 June and was found at 1850 h inside another burrow containing a freshly laid nest in the apron (Table 1). A second video-recorded resident female abandoned a nesting attempt at her burrow after hitting a large root, and, based on subsequent camera observations at her burrow, likely nested elsewhere. A third video-recorded resident tortoise that nested at her burrow had only moved to that burrow within the previous 10 days.

I documented seven females each abandon a single nesting attempt prior to oviposition, one for an unknown reason, one perhaps in response to an approaching rainstorm, and the remainder apparently in response to a root in the nest cavity (one female), researcher disturbance (one female), and conspecifics (three females; Table 1). Conspecifics also temporarily disrupted the nesting activities (nest covering and manicuring) of two females that had oviposited earlier in the day (Table 1).

Nesting behavior

Nesting could be divided into five stages: circling and/or digging a shallow depression, digging the egg cavity, ovipositing, covering the nest, and manicuring the

nest area. Time spent in different stages differed ($H = 25.8$, $df = 4$, $P < 0.001$; Table 2). Females exhibited wide variation in the first nesting stage, circling and/or digging a shallow depression, and not all females performed this stage. Four females initiated nesting by circling on their center axis at the prospective nest location. Four other tortoises initiated nesting by both circling and constructing a shallow depression. However, one of these tortoises started one nesting attempt by circling and digging a shallow depression, but started another by immediately excavating a nest cavity. Similarly, a ninth tortoise basked on her burrow apron for 29 min without changing position and transitioned directly to excavating a nest cavity in the same location without first circling or digging a shallow depression. Most females lowered their head to the ground a small number of times before nesting, but this behavior was minimal during this stage. Mean time spent circling and/or digging a shallow depression was 7.0 min (range = 0.0–15.0 min, $n = 8$: one female excluded because she abandoned her attempt during this stage; Table 2).

When excavating nest cavities, females faced away from, rather than toward, burrow entrances more often than expected by chance (nine of nine facing away; binomial test: $P = 0.004$) and used their hindlimbs to remove soil. As time progressed, individuals lifted up slightly on their forelimbs as if to reach deeper into the cavity with the hindlimbs. In some instances, females kicked excavated soil into and behind burrow entrances. Mean time to excavate the nest cavity was 40.3 min (range = 25.3–59.5 min, $n = 6$).

After constructing a nest cavity, tortoises oviposited immediately. All females laid eggs without interruption while facing away from the burrow entrance. Ovipositing

females pumped their heads in and out of their shells. Due to tortoise and camera positioning, it was only possible to see into the egg chamber during one oviposition event. In that instance, the female used a hindlimb multiple times to manipulate eggs within the nest cavity. Two ovipositing females exhibited extensive frothy saliva or mucus discharge from the mouth and nares. One of these females also had discharge from the eyes. Mean time to oviposit was 10.9 min (range = 7.2–16.1 min, $n = 6$; Table 2).

After ovipositing, females immediately covered nests. Females initially used their hindlimbs to cover the eggs, while continuing to face away from the burrow entrance. Forelimbs remained planted stationary on the ground from when tortoises initiated cavity excavation until individuals nearly completed covering the nest using their hindlimbs, at which point the forelimbs were employed to finish the task. Mean time to cover nests was 15.3 min (range = 10.0–22.0 min, $n = 6$). Initiation of nesting through final covering of eggs averaged 73.8 min (range = 52.1–112.6 min, $n = 6$; Table 2).

After covering nests, females ($n = 6$) extensively manicured the nest area, burrow entrance, and surrounding burrow apron. Manicuring females kicked soil and other materials out of burrow entrances, some of which had accumulated during excavation of the nest cavity, and roughed up soil on the burrow apron using their forelimb claws. On average, females repeated the behavior of entering (or partially entering) burrows and kicking soil out 19.7 times (range = 11–30, $n = 6$ tortoises) and spent about 150.5 min (range = 101.8–207.5 min, $n = 6$; Table 2) engaged in manicuring activity during daylight hours following nesting. Manicuring females also repeatedly nuzzled the ground. On average individuals lowered their noses toward the ground at least 106 times (range = 22–200, $n = 6$) during manicuring activities before retreating into burrows by dark. Due to

camera angles, vegetation, and low video frame rate (0.2 frames/s), I likely undercounted this behavior. At least four of six females emerged from burrows much earlier than usual the morning following nesting and continued some manicuring activities. Observations on days following nesting were unavailable for two females. In one case, I installed protective wire mesh over the nest (Radzio et al. 2017; Appendix 2) soon after it was laid, and the female left shortly thereafter and did not return. In the other case, the female overnighted in the burrow after nesting, but additional video was not recorded at that burrow.

Conspecific interactions during nesting

I documented interactions between nesting females and conspecifics in five of 12 video-recorded nesting attempts ($n = 6$ total interactions, one nesting event included two interactions). In each instance that the interaction occurred before eggs were laid ($n = 3$), the female abandoned the nesting attempt (Table 1). In one interaction, a visiting female (Visitor 1) arrived at a burrow containing a male and immediately began to nest on the burrow apron, but abandoned the attempt soon thereafter, apparently when the male emerged (Table 1). Similarly, another female (Resident 3) immediately abandoned nest site preparation at her burrow when a male visited. She nested in the same location alone 2 days later. A third female (Visitor 3) visited a burrow containing another female and started to dig a cavity, but abandoned the attempt when the resident emerged (Fig. 1A–B). The two tortoises interacted on the burrow apron before both left the area. On the following day, when the burrow was vacant, the visiting female returned and nested. Visiting males disturbed two other females after they oviposited. A male visited a female

(Resident 1) as she covered her nest and vigorously tried to mate with her, immediately biting, mounting, and ejaculating/ urinating on her shell. She was unreceptive, and at one point, the aggressive male overturned in the partially filled in nest (Fig. 1C–D). Later that day, another male visited, and disrupted nest manicuring activity, but may have successfully mated with the now more receptive female. A male also visited another female (Resident 6) as she manicured a nest she laid earlier in the day and distracted her from this activity for about 30 min (Table 1).

Additional female-female interactions at burrows

Cameras documented 12 visits by females to female-occupied burrows outside obvious nesting activity (Table 3). Observations involved at least seven females. Visiting and resident females interacted in 11 of 12 visits (Table 3). In the lone visit without an interaction, the resident tortoise remained inside the burrow out of camera view while the visitor briefly inspected the burrow entrance. Ten of the 11 interactions involved at least one gravid tortoise (Table 1). Ten interactions lasted fewer than 20 min. One interaction, which included overt aggression, involved tortoises sharing a burrow for at least 2 days.

Six of 11 interactions involved overt aggression in which at least one female rammed the other tortoise with her the gular protrusion and/or carapace. At least five females exhibited such overt aggression toward another female. In three of the five remaining interactions without overt aggression, a female was initially at the surface and positioned itself (in two instances quite quickly) in the burrow entrance as if to prevent the other female from entering. In the remaining two interactions without overt aggression, a female was already in the burrow entrance, facing inside, and responded to

another female on the burrow apron either by turning sideways in the burrow entrance or turning to face completely outward.

Additional female-male interactions at burrows

Cameras documented 49 visits by males to females ($n = 10$) at their burrows outside of obvious nesting activity. A female ($n = 9$) was at, or emerged to, the surface during 40 of these visits. Males attempted to mate with females in at least 38 of 40 (95.0%) such encounters (Table 3).

Mating attempts often proceeded by: 1) the male headbobbing toward (and sometimes biting) the female; 2) the male backing away from a female; 3) if receptive, the female advancing toward the male; 4) the male continuing to headbob toward (and sometimes biting) the female; 5) the female turning around to face away from the male (and toward the burrow entrance); 6) the male rapidly mounting the female; 7) the female moving toward and into the burrow, which would cause the male to become dislodged or voluntarily dismount before copulating; and 8) often repeats of this sequence once the female reemerged from the burrow. At other times, males initiated mating attempts by immediately mounting and, sometimes, biting females. Males regularly mounted females from ineffective copulatory positions, such as from the front or side of the carapace. Rapid mounting often led to ejaculation or urination onto female carapaces or onto the ground. In at least one instance, a male ejaculated or urinated onto the ground very soon after arriving, before even mounting the female.

Males mounted females as many as six times per interaction with obvious mating attempt (mean = 1.8 mounts), but appeared to copulate only in up to five of 69 (7.2%)

total mounting attempts, or five of 38 (13.2%) interactions involving obvious mating activity (Table 3). In these potentially successful copulations, which were distributed evenly among five females, males remained mounted longer, made deep head thrusts, dismounted females voluntarily, and left immediately thereafter. Similar to ovipositing females, several males expelled mucus or saliva from their nares during potentially successful copulation events. Although male mating attempts often were aggressive, I did not observe females exhibiting overt aggression (i.e., ramming, pushing) toward males.

Female-large juvenile interactions at burrows

Large juveniles (about 15 cm carapace length) briefly visited burrows containing adult females on five occasions. On two occasions, the resident female emerged from the burrow and rammed the smaller tortoise. On one occasion, a large juvenile visited a burrow while the resident female was inside and a visiting female was about to initiate a nesting attempt on the burrow apron. The large juvenile and the visitor interacted, but the interaction did not include overt aggression. On two other occasions, large juveniles visited burrows containing adults, but the adult did not emerge from the burrow nor was observed in the entrance (Table 3).

Discussion

Nesting behavior

Similar to reports for other Gopher Tortoise populations (Landers et al. 1980, Butler and Hull 1996, Epperson and Heise 2003, Hammond 2009, Lamb et al. 2013), females at this site frequently, perhaps predominantly, nest at burrow aprons. Gopher

Tortoises in Georgia nest up to once annually (Landers et al. 1980), and at least 6 of 10 resident females in this study exhibited nesting activity at burrow aprons. I also add to other reports indicating that females do not nest exclusively at their own burrow aprons but may select nest sites from among multiple burrows, including those of juvenile tortoises, and other bare ground areas (Landers et al. 1980, Lamb et al. 2013, Radzio et al. 2017). My observations indicate that at least five females exhibited nesting activity away from their burrows, one on two occasions (Table 1). Additionally, another tortoise that nested at her burrow had only moved to that burrow within the previous 10 days. Although quantitative data are limited, tortoises may select among multiple potential nest sites on the basis of vegetative cover, soil composition, and thermal environment (Landers et al. 1980, Diemer and Moore 1993, Smith 1995, Lamb et al. 2013).

In some diurnal turtle species, high daytime temperatures constrain nesting to night hours and other cooler times of day (Spotila and Standora 1985). Video-recorded nesting observations distributed rather uniformly between 1000 and 1800 h, but sample size was low. Given that females required on average more than an hour to nest, they should avoid nesting in open habitats during very hot weather. In this study, two video-recorded females secreted large amounts of mucus or frothy saliva when thrusting their heads in and out of shells during oviposition. Although it is possible that this discharge represented a physiological response to thermal stress (Douglass and Layne 1978, Johnston 1996), I was unable to unambiguously assess heat loads experienced by these nesting tortoises. Additionally, discharge may have reflected symptoms of upper respiratory disease (McLaughlin et al. 2000) or simply represented a feature of nesting that sometimes occurs in healthy individuals. Notably, some males exhibited similar

discharge when performing deep head thrusts during potentially successful mating attempts.

Gopher Tortoises in this study exhibited many typical turtle nesting behaviors, including nest site preparation (Ehrenfeld 1979), but this activity was variable even within individuals. Kenefick (1954) reported that a captive Gopher Tortoise began nesting by swinging its body in a circle and digging a shallow depression with its forelimbs. I observed similar behavior in most nesting tortoises, but several times females started nesting by immediately digging an egg cavity with their hindlimbs. Females engaged in little to no ground-nuzzling behavior before starting to nest (Morjan and Valenzuela 2001 and references therein), but as described below, engaged in this behavior extensively when manicuring the nest area following oviposition. Typical of most chelonians (but see Kuchling 1993), individuals in this study used their hindlimbs to both excavate and initially cover the egg cavity (Ehrenfeld 1979). Tortoises required considerable time to excavate the nest cavity, but after doing so, immediately laid eggs, on average within 11 min. By ovipositing quickly and immediately covering the nest, tortoises may reduce depredation risk to themselves and their eggs and also prevent nest substrate from losing excessive moisture. Unlike as reported for captive Agassiz's Desert Tortoises (*G. agassizii*; Lee 1963) and free-ranging Texas Tortoises (*G. berlandieri*; Auffenberg and Weaver 1969, Rose and Judd 2014), I did not observe Gopher Tortoises urinating on nests. However, due to camera angles and tortoise orientations, I could only see into the nest cavity in one video.

Tortoises always oriented facing away from burrow entrances while excavating the nest cavity (Butler and Hull 1996), ovipositing, and covering eggs. The soil in front of

burrows often slopes down toward the burrow entrance. Facing upslope may allow females to reach deeper into the nest cavity with their hindlimbs, deposit excavated soil downhill, and detect potential predators (Butler and Hull 1996) or visiting conspecifics more easily because the head remains elevated and out of the burrow. This orientation often also results in excavated soil being scattered behind the back edge of the burrow entrance, a sign that can be used by investigators to locate nests (Matt Hinderliter, pers. comm.).

After covering nests, females manicured nest areas, perhaps having the effect of reducing egg depredation, which can be very high in Gopher Tortoise populations (Landers et al. 1980, Smith et al. 2013). Kenefick (1954) reported that after covering her nest, a captive Gopher Tortoise “walked back and forth over the nest area and brushed it lightly with the nails of her front feet” for a short period. My observations indicate that wild females engage in extensive nest manicuring intermittently throughout the day, or even days, following nesting and suggest that females also disguise nests by kicking soil out of burrows onto burrow aprons. All females intermittently kicked soil out of the burrow and onto the apron throughout the afternoon following nesting. Using their forelimbs, they also roughed up soil over a portion of the burrow apron. By excavating soil from burrows, tortoises can remove material that accumulated in burrow entrances during nest cavity excavation and create the visual appearance of a burrow that has been dug out or cleaned by a tortoise, rather than one that contains a nest in its apron. It is also possible that by kicking soil out of burrows, which frequently contain tortoise feces, tortoises mix in odors from the burrow to the nest area, and that this may confuse potential predators that locate turtle nests via olfactory cues, including volatiles released

from disinterred soils (Buzuleciu et al. 2016). Manicuring females engaged in extensive ground nuzzling, a common turtle nesting behavior hypothesized to play a role in nest site selection either via detection of thermal or olfactory cues (Morjan and Valenzuela 2001). Therefore, it is notable that Gopher Tortoises ground nuzzled extensively after, but very little or not at all before nesting. A recent study of Painted Turtles (*Chrysemys picta*) suggests females can detect nests of conspecifics via olfactory cues (Iverson et al. 2016), and I speculate that Gopher Tortoises use ground nuzzling behavior and olfactory senses to guide efforts to disguise nest odors. Disguising nest odors for even a few days may be highly beneficial because turtle nests may be at greatest risk of depredation early in incubation (Congdon et al. 1983, 1987). Interestingly, a camera documented a Gray Fox (*Urocyon cinereoargenteus*) visit a burrow apron containing a nest that had been laid fewer than 6 h earlier. The female, who was sitting atop her nest when the fox arrived, quickly hid inside. The fox left the burrow seconds later without disturbing the nest. Although not observed in this study, recent accounts suggest that some female Gopher Tortoises defend nests against potential predators (Grosse et al. 2012, Dziadzio and Smith 2015). Nest defense is also documented in several western *Gopherus* species, including ones that often nest inside burrows (Roberson et al. 1985, Turner et al. 1986, Agha et al. 2013 and references therein), where predation risk to defending females might be limited because large predators cannot enter narrow burrows.

Conspecific interactions

My observations suggest that, in addition to abiotic factors (Landers et al. 1980, Diemer and Moore 1993, Smith 1995, Lamb et al. 2013), conspecific interactions may

directly or indirectly influence where individual females nest. In this study population, approximately one-quarter to one-half of adult-sized tortoise burrows are used by an adult tortoise at any given time (Burke 1989, Guyer et al. 2012), and tortoises spend virtually all of their nighttime hours and nearly all of their daytime hours at burrows (T. Radzio, unpubl. data). In each of the three instances where I documented a female attempting to nest in the presence of a conspecific, the female abandoned the attempt. It is likely more difficult for a female to nest at a burrow occupied by an adult conspecific because as my observations of tortoise interactions indicate, if the burrow contains a female, she may be aggressively pushed or rammed, or if it is occupied by a male, she may be aggressively bit and/or mounted (Douglass 1986, Johnson et al. 2007, Guyer et al. 2014; this study). Even if she manages to oviposit, her eggs could be inadvertently trampled and broken during interactions with the conspecific. Any of these possibilities could explain why females abandoned nesting attempts following interactions with conspecifics.

To adversely influence reproductive success or offspring phenotype, conspecific constraints on nesting activity must affect nest characteristics such as depredation risk or incubation conditions. Although Gopher Tortoise nests at this site exhibit substantial variation in hatching success (0–100%) and oviposition-to-hatchling emergence times (96–128 d; Radzio et al. 2017; Appendix 2), potentially reflecting underlying variation in incubation conditions, my data do not assess whether social interactions affect reproductive outcomes. However, my observations do suggest that, if Gopher Tortoises exhibit nest site philopatry, it could be somewhat obscured by conspecific constraints on where females nest.

I document apparent burrow competition involving gravid female tortoises, avoidance responses by nesting females to male and female conspecifics, and other social interactions outside of nesting in an old-growth Longleaf Pine forest that suggest movements and nest-site choices of female Gopher Tortoises may be influenced by conspecific interactions. Old-growth Longleaf Pine forest is hypothesized to be one of the primary ancestral habitats of Gopher Tortoises (Guyer and Hermann 1997), but Gopher Tortoises inhabit a variety of environments, including less productive Longleaf Pine ecosystems and barrier islands where ground cover is less dense. At a site characterized by many unvegetated areas, Smith (1995) documented extensive nesting activity by Gopher Tortoises away from, but very little at, burrow aprons. Therefore, my observations may serve as a reference for how social interactions influence the nesting ecology of Gopher Tortoises in a portion of their natural environment, particularly where tortoises occur in high densities and nest extensively on burrow aprons.



Figure A-1. Images from time-lapse video camera stations at two Gopher Tortoise (*Gopherus polyphemus*) burrows in southwestern Georgia, USA. (A-B) Female Gopher Tortoise (Visitor 3; left) visits the apron of a burrow containing a female conspecific (Resident Female 2; right) and starts to excavate a nest cavity. Shortly thereafter, the resident female emerges; the visitor stops digging; the tortoises interact; and the visitor leaves. Later that day, the resident female also left. On the following day, the visiting tortoise returned and successfully nested at the vacant burrow. (C-D) Visiting male aggressively mounting a female (Resident Female 1) while she attempts to cover or manicure her nest, which she laid minutes before. The male overturned while attempting to mount the female.

Table A-1. Nesting observations, minimum number of nesting attempts away from own burrow (NA), minimum number of female-female interactions at potential nesting sites (burrows) 10 days preceding nesting (FFI), and minimum number of conspecific disruptions to nesting activity (includes disruptions during nest manicuring activity; CD) for individual Gopher Tortoises (*Gopherus polyphemus*) from a site in southwestern Georgia, USA, based on video recordings at burrow aprons. Asterisks indicate possible nesting activity away from own burrow.

Female	Nesting Observations	NA	FFI	CD
Resident 1	5 June 2013, 1146–1207 h: Attempted to nest at own burrow. Abandoned attempt, perhaps due to approaching rainstorm. 8 June 2013, 1009–1117 h: Nested successfully at own burrow. Two visiting males disrupted nest covering and manicuring.			2
Resident 2	7 June 2013, 1550–1622 h: Abandoned nesting attempt at own burrow. Large root observed in abandoned cavity. 7–14 June 2013: No further nesting activity at this burrow. Female possibly nested away from own burrow.	1*		
Resident 3	2 June 2013, 1154–1159 h: Abandoned nesting attempt at own burrow when male visited. 4 June 2013, 1047–1240 h: Nested at own burrow.		2	1
Resident 4	9 June 2013, 1011 h: Left own burrow. Tracked to new burrow at 1850 h. Apron of new burrow contained fresh nest.	1*	1	
Resident 6	5 June 2013, 1011–1103 h: Nested at own burrow. Visiting male disrupted nest manicuring activity.		2	1
Resident 8	9 June 2013, 1447–1615 h: Nested at own burrow.		6	
Resident 9	7 June 2013, 1344–1441 h: Nested at own burrow.			
Visitor 1	25 May 2013, 1418–1428 h: Visited male-occupied burrow. Started to nest, but abandoned attempt.	1		1
Visitor 2	1 June 2013, 1425–1432 h: Visited Resident Female 8's burrow while resident was away. Started nesting, but abandoned attempt.	1	2	
Visitor 3	12 June 2013, 1736–1739 h: Visited Resident Female 2's burrow. Abandoned nesting attempt when resident emerged from burrow. Both tortoises abandoned burrow. 13 June 2013, 1440–1545 h: Returned and nested at vacant burrow.	2	1	1

Table A-2. Time spent by female Gopher Tortoises (*Gopherus polyphemus*) from a site in southwestern Georgia, USA, in each nesting stage and total time required to construct a nest (excluding nest manicuring activity). Different letters next to mean values denote significantly different time spent in stages (Bonferroni-corrected Mann-Whitney U tests). Manicuring activity refers to manicuring activity on the day of oviposition and does not include additional nest manicuring on subsequent days.

Activity	Mean (min)	Range (min)	N
Circling/Digging Depression	7.0 (a)	0.0–15.0	8
Digging Egg Cavity	40.3 (b)	25.3–59.5	6
Oviposition	10.9 (a)	7.2–16.1	6
Covering Egg Cavity	15.3 (a)	10.0–22.0	6
Total Time to Construct Nest	73.8	52.1–112.6	6
Nest Manicuring	150.5 (c)	101.8–207.5	6

Table A-3. Conspecific encounters (multiple individuals observed together) of Gopher Tortoises (*Gopherus polyphemus*) from a site in southwestern Georgia, USA, outside obvious nesting activity, but during the nesting season. Number of encounters (NE), encounters with interactions (EI), interactions with aggression (IA), interactions with mating attempts (IMA), and interactions with potential matings (IPM; interaction types defined in Materials and Methods). In 6 of 11 female-female interactions, at least one female rammed the other. In each of the five remaining potentially aggressive interactions, a female blocked the burrow entrance.

Encounter Type	NE	EI	IA	IMA	IPM
Female-Female	11	11	6-11	-	-
Female-Male	40	39	-	38	5
Female-Large Juvenile	3	3	2	-	-

Appendix B. Hatching Success and Other Reproductive Attributes of Gopher Tortoises in Southwest Georgia

Abstract

Broad variation in egg hatching success observed in gopher tortoise (*Gopherus polyphemus*) populations led me to investigate hatching success and other reproductive attributes within a unique, high-quality site in the eastern portion of the species' range. I documented for the first time use of juvenile tortoise burrows as nest sites, a mean clutch size of 5.9 eggs, long oviposition-to-hatchling emergence times (96–128 d), and 73% hatching success for predator-protected eggs. Although consistent with previous reports of greater hatching success in eastern gopher tortoise populations than in western ones, hatching success at the eastern site was on the low end of values from other eastern populations, possibly reflecting above average rainfall during this study.

Introduction

Gopher tortoises (*Gopherus polyphemus*) inhabit dry upland areas within the Coastal Plain of the southeastern United States from Louisiana to South Carolina. Habitat loss and alteration threaten gopher tortoises range-wide (Auffenberg and Franz 1982, U.S. Fish and Wildlife Service [USFWS] et al. 2013), but in some populations, low egg-hatching success represents another threat to population stability (Epperson and Heise 2003, Ennen et al. 2010, Noel et al. 2012). When protected from predators, naturally incubated eggs from eastern populations (east of the Tombigbee River) exhibit high hatching success (67–97%; Landers et al. 1980, Smith 1995, Butler and Hull 1996).

However, for uncertain reasons, predator-protected eggs from western populations, where gopher tortoises are listed as Threatened under the Endangered Species Act of 1973 (USFWS 1987), exhibit low hatching success (18%–47%; Epperson and Heise 2003, Hammond 2009, Noel et al. 2012).

To help document variation in gopher tortoise reproductive potential, I investigated egg hatching rates in an eastern population of gopher tortoises inhabiting a longleaf pine forest (*Pinus palustris*) in southwestern Georgia. The biologically diverse longleaf pine ecosystem is hypothesized to be a major ancestral habitat of the gopher tortoise (Guyer and Hermann 1997) but today is one of the most endangered ecosystems in North America (Noss et al. 1995, Van Lear et al. 2005). The study site, Arcadia Plantation, was composed of mature longleaf pine forest and included the Wade Tract, one of only a few remaining old-growth longleaf pine stands. I was particularly interested in determining hatching rates of tortoise eggs at this site given its potential reference and conservation value and because tortoise egg-hatching success had not been previously investigated at this eastern location.

Materials and Methods

I studied tortoise nests on Arcadia Plantation (30°45' N, 84°0' W), a 957-ha property located in Thomas County in southwestern Georgia, USA. Arcadia Plantation is dominated by mature (> 80 yrs) longleaf pine forest and is managed using frequent prescribed burns (< 2-yr return intervals). The study site included the Wade Tract, an 80-ha, old-growth research area that has never been heavily cut and contains many old (> 200 yrs) trees (Platt et al. 1988). Ground cover on both Arcadia Plantation and the Wade

Tract was dominated by wiregrass (*Aristida stricta*), oaks (*Quercus* spp.), and a diverse assemblage of other native plants (Ambrose 2001). Soils at the site are a complex mosaic of sand and clay (NRCS Web Soil Survey 2016).

I located nests in May and June 2013 primarily by looking for evidence of nesting at tortoise burrow aprons (area of bare soil located in front of burrows), such as sand kicked behind burrow entrances (M. Hinderliter, pers. comm.). I also searched for signs of nests at juvenile tortoise burrows and in other open areas. Additionally, I found some nests by setting time-lapse video cameras at adult burrows thought to contain female tortoises or to be potential nesting sites. Although I did not check potential nest sites daily, all discovered nests appeared to have been recently laid. I determined exact oviposition dates for 6 nests from video observations of nesting tortoises and for a seventh nest from a combination of video and visual observations. Upon finding an intact nest (one that was not damaged by a predator), I buried hardware cloth above the nest just below the ground surface, and, in most cases, secured the cloth using stakes to deter potential predators from excavating eggs. I did not handle or move eggs, but did briefly partially uncover and examine (without moving) the top eggs of 3 nests later in the incubation period.

In late July, I replaced nest protectors with cages that would continue to protect nests yet also allow hatchlings to emerge and be retained. I constructed nest cages ($40 \times 40 \times \sim 6$ cm [width $\times$ length $\times$ height]) using hardware cloth, staked them into the ground above nests, and buried their edges in the substrate. I affixed a small piece of burlap to one or more sides and corners of each nest cage to provide a small shaded area for emerging hatchlings (Epperson and Heise 2003, Noel et al. 2012). Beginning in early

August, I visited each nest site daily to check for hatchlings. When hatchlings began to emerge in early September, I increased visit frequency.

When emerged hatchlings were encountered, I removed them from the nest cage. In some instances, I also removed hatched siblings if they had resorbed the yolk sac to prevent potential depredation by red imported fire ants (*Solenopsis invicta*; Landers et al. 1980, Smith 1995). In one case, I loosened hard-packed clay from above a nest where hatchlings could be heard trying to scratch through to the surface. These hatchlings had already dug nearly to the surface and likely would have emerged without assistance by the following day when heavy rains loosened soils. In late October, > 2 weeks later in the year than when hatchlings at this latitude are known to emerge (Landers et al. 1980, Smith 1995, Butler and Hull 1996, Epperson and Heise 2003, Noel et al. 2012), I excavated all nests to determine the number of remaining live hatchlings, dead hatchlings, or unhatched eggs.

I compared monthly rainfall totals at the site in 2013 (recorded by a mobile weather station) with historical monthly rainfall totals recorded at a Global Historical Climatology Network weather station (Station ID: GHCND:USC00098666) located ~ 20 km northeast of my study site. Complete data were available for most years during 1893–2012 from the National Oceanic and Atmospheric Administration National Climate Data Center (available at ncdc.noaa.gov, accessed 4 March 2015). Summary statistics are presented as means $\pm$ 1 standard deviation.

Results and Discussion

From 28 May to 16 June 2013, I located 20 recently laid gopher tortoise nests. Predators destroyed 2 nests and disturbed 1, leaving 17 nests for monitoring. These 17 nests were distributed among the following locations: aprons of adult tortoise burrows (13); in a dirt road (1); in the apron of a juvenile gopher tortoise burrow (1; burrow entrance width ~ 13 cm); next to a cut tree stump (1); and in a sparsely vegetated area not directly associated with a tortoise burrow (1). Gopher tortoises frequently nest in the aprons of adult burrows (Landers et al. 1980, Butler and Hull 1996, Epperson and Heise 2003, Noel et al. 2012; but see Smith 1995), but observations at the study site indicate they also nest in the aprons of juvenile tortoise burrows. I located 2 tortoise nests at juvenile burrow (burrow entrance widths ~ 13 and ~ 18 cm) aprons at this site in previous years while setting bucket traps to capture young tortoises (T. Radzio, unpubl. data).

Female tortoises potentially use juvenile burrow aprons as nest sites for some of the same reasons that they nest at adult burrow aprons. For example, relative to surrounding areas, both adult and juvenile burrow aprons contain less vegetation that can interfere with nest excavation (Landers et al. 1980; Appendix 1). Additionally, these bare sites are likely warmer than surrounding microhabitats, which may be important for successful incubation. Burrow aprons are also located next to a refuge that hatchlings can temporarily occupy following nest emergence (Radzio et al. 2009). To avoid damaging tortoise eggs, investigators setting bucket traps in front of burrows of juvenile tortoises during the incubation season are advised to check carefully for nests.

Hatchlings emerged from 2 September until 10 October 2013. Peak hatchling emergence occurred during 17–27 September, when the first hatchlings emerged from 10 of 16 nests. For 16 nests that produced hatchlings, mean time from discovery of a nest to emergence of the nest's first hatchling was 108.3 ± 8.7 d, but varied by > 4 weeks (range = 96–128 d). For 7 of these nests with known oviposition dates, mean time from oviposition to emergence of the nest's first hatchling was 112.6 ± 10.5 d (range = 97–128 d). Mean and maximum oviposition-to-hatchling emergence times were longer, generally substantially, than reported for other northern gopher tortoise populations (Landers et al. 1980, Smith 1995, Butler and Hull 1996, Noel et al. 2012). I hypothesize that above average rainfall resulted in lower incubation temperatures, leading to the long hatchling-emergence times observed in this study. Additionally, the nest with the longest hatchling-emergence time (128 d) was in a relatively shaded burrow apron.

Mean hatching success of individual nests was $69.4\% \pm 34.7\%$ ($n = 17$). Seventy-four of 101 (73%) eggs hatched, which is on the low end of hatching rates reported for other eastern gopher tortoise populations (67%–97%; Landers et al. 1980, Smith 1995, Butler and Hull 1996), but still higher than reported for tortoise eggs from western sites (18%–47%; Epperson and Heise 2003, Hammond 2009, Noel et al. 2012). Sixteen of 17 nests produced > 1 live hatchling, and 7 of 17 nests exhibited complete hatching success. Of 27 eggs that failed to hatch, only 4 (15%) contained dead hatchlings that were almost fully developed (i.e., scutes present). The low proportion of late-stage egg failures in this study is consistent with the results of Butler and Hull (1996), the only study to report development stage of failed eggs for an eastern population of gopher tortoises. However, late-stage failures accounted for up to 53% of unhatched eggs in western populations

(Epperson and Heise 2003, Hammond 2009, Noel et al. 2012). Mean clutch size was 5.9 ± 1.8 (range = 3–9 eggs).

Although my observations are consistent with previous reports of greater hatching success in eastern gopher tortoise populations than in western ones (Epperson and Heise 2003, Hammond 2009, Noel et al. 2012), hatching success at this eastern site was on the low end of values reported from other eastern populations (Landers et al. 1980, Smith 1995, Butler and Hull 1996). Notably, rainfall was much higher than average during much of the incubation period in this study. Total July rainfall at the study site (25.7 cm) was greater than was observed at a nearby weather-sampling site in all but 8 of 85 preceding years and was also high in late June. Very high soil moisture can reduce hatching success of turtle eggs (Ragotzkie 1959, Kraemer and Bell 1980), even when soils are not fully saturated with water (McGehee 1990). Future work should examine whether egg hatching success at this site is greater in drier years.

VITA

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EDUCATION

Ph.D., Environmental Science , Drexel University, Philadelphia, PA	2017
M.S., Biology , Ohio University, Athens, OH	2004
B.S., Biology and Chemistry , Longwood University, Farmville, VA	2001

PROFESSIONAL EXPERIENCE

Graduate Teaching Assistant in Biology , Drexel University	2010-17
Automated Telemetry Coordinator , USFWS	2010
Supervisory Gopher Tortoise Research Biologist , US Army CERL	2006-10
Fisheries Field Technician , James Madison University	2004
Diamondback Terrapin Field Assistant , Ohio University, USGS	2001-05
Graduate Teaching Assistant in Biology , Ohio University	2001
Fisheries Field Technician , Virginia Institute of Marine Science	2000
NSF-REU Marine Biology Intern , Western Washington University	1999

AWARDS AND GRANTS

Student Presentation Awards

Gopher Tortoise Council	2013, 2014, 2017
Turtle Survival Alliance	2011, 2014
Ecological Society of America Mid-Atlantic Chapter	2014
American Society of Ichthyologists and Herpetologists	2015
Claudio Elia Fellowship in Environmental Science , Drexel University (\$7,500)	2014
Funds for Tortoise Release Study , US Army (\$3,000)	2014
McLean Fellowship in Environmental Science , Drexel University (\$12,500)	2013
Research in Herpetology Grant , Chicago Herpetological Society (\$1,000)	2012
Biodiversity Conservation Research Fund , The Nature Conservancy (\$3,993)	2002

SELECTED PUBLICATIONS

Radzio TA and MP O'Connor. 2017. Behavior and temperature modulate a thermoregulation-predation risk trade-off in juvenile gopher tortoises. *Ethology* 123:957-965.

Radzio TA, J Cox, JR Spotila, and MP O'Connor. 2016. Aggression, combat and apparent burrow competition in hatchling and juvenile gopher tortoises. *Chelonian Conservation and Biology* 15:231-237.

Radzio TA, JA Smolinsky, and WM Roosenburg. 2013. Low use of required terrapin bycatch reduction devices in a recreational crab pot fishery. *Herpetological Conservation and Biology* 8:222-227.

Radzio TA and WM Roosenburg. 2005. Diamondback terrapin mortality in the American eel pot fishery and evaluation of a bycatch reduction device. *Estuaries (presently Estuaries and Coasts)* 28:620-626.