Anthropogenic Effects on Freshwater Turtles Across Life Stages

by

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Abstract

Turtles are one of the most imperiled vertebrate taxa and in light of rapid global urbanization, habitat destruction, and climate change it is crucial to quantify relationships between anthropogenic change and turtle responses. In my dissertation, I explore how various anthropogenic impacts influence freshwater turtle populations at multiple life history stages.

In Chapter 1, I investigate the impact of urbanization on nest site choice among turtles in the family Emydidae. Nest site choice can have important implications for embryo and hatchling survival, and ultimately, population persistence. First, I determined whether turtles selected nest sites based on a range of microhabitat variables by comparing maternally selected natural nests to randomly chosen artificial nests. Additionally, I compared nest site choice across areas with different levels of human disturbance. Natural nests had less variance in canopy openness and average daily mean and minimum temperature than artificial nests, but microhabitat variables were similar across differing levels of disturbance. Overall, these results show that turtles are not adjusting their choices of nest microhabitat when faced with anthropogenic change, suggesting that preserving certain natural microhabitat features will be critical for populations in human-disturbed areas.

In Chapter 2, I explore how early life environments, that can potentially be altered by climate change, influence hatchling pond slider (*Trachemys scripta*) performance. Specifically, I determined how incubation temperature and overwintering environment impact hatchling righting response. I incubated eggs at five constant temperatures (24°C, 26°C, 28°C, 30°C, 32°C). After hatching, turtles were distributed among housing

treatments that simulated either overwintering in a terrestrial nest or an aquatic environment. In the spring following hatching, I conducted righting response trials, which are frequently used performance measures that may be associated with coordination and neuromuscular function. Results showed that incubation temperature, overwinter environment, and their interaction significantly influenced hatchling phenotypes and righting performance. Aquatically overwintering hatchlings that were incubated at cooler temperatures righted themselves faster than those incubated at warmer temperatures. This relationship did not exist in terrestrially overwintering hatchlings, which had faster overall righting times that were not affected by incubation temperature. Altogether, these findings highlight the sensitivity of turtles to environmental alterations, as early life conditions are crucial in determining hatchling survival.

In Chapter 3, I investigate the relationship between urbanization and sex ratios in pond sliders (*Trachemys scripta*). Increased road density and other variables associated with urbanization can have profound impacts on species that must move across the landscape. Previous work has shown that male biases in many populations of freshwater turtles are related to increased road mortality of females as they travel over land to build a nest. However, analyses related to much of this work focus on sex ratios based on sampled numbers of individuals, introducing the potential for biases. I set turtle traps at five ponds along an urbanization gradient in Lee, Macon, and Chambers counties, Alabama from 2019 to 2025. I analyzed adult sex ratios in relation to a number of variables associated with urbanization. In addition, I built capture-mark-recapture models to estimate sex-specific population sizes at each site. I present results of analyses using both sampled proportions of males and females, as well as proportions derived from population size estimates. I found an unexpected negative relationship of urbanization with the proportion of males in the population. However, differences in human housing density and impervious surfaces at our most urban sites may be driving these results, highlighting how making distinctions between different landcover variables associated with urbanization is vital in understanding human impacts on populations. In addition, I show that there are drastic differences in the statistical significance of results between analyses using sampled versus estimated sex ratios. This finding highlights the importance of obtaining accurate estimates of male and female population sizes when making inferences related to urbanization effects on sex ratios.

Collectively, my dissertation documents turtle responses to anthropogenic change at various life stages that are important to population persistence. In addition to illustrating the unique responses that may occur at each stage, my dissertation sheds light on potential management strategies that could improve outcomes for urban turtle populations.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this dissertation, no Artificial Intelligence (AI) tools were used.

Digital Accessibility Use Disclosure Statement

In the preparation of this dissertation, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Office Accessibility Checker. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

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Introduction

The growing human population and its alteration of natural habitats has affected nearly every ecosystem on the planet. Human-induced changes across the globe impact wildlife populations dramatically and in a myriad of ways. Habitat destruction can cause species declines and extinctions, while habitat fragmentation can drastically alter movement patterns and pose dangers to mobile species, and overexploitation can rapidly decimate populations (Andrén, 1997; Czech et al., 2000; Rosser & Mainka, 2002). The increase of CO_2 emissions and the resultant changes in climate and weather patterns worldwide has extreme impacts on aspects of biology ranging from physiology, reproduction, and development, to phenology and migration patterns (Bellard et al., 2012).

Urbanization, as a specific category of anthropogenic impact, is one of the leading causes of species endangerment (Czech et al., 2000; Mcdonald et al., 2008), and has gained increased attention in recent years in reference to its impacts on wildlife (Collins et al., 2021). Defined as a high concentration of human infrastructure that occurs in highly populated areas, urbanized areas are rapidly increasing across the planet. Urban environments typically experience highly altered habitats (Faulkner, 2004; Shochat et al., 2004), increased temperatures (Peng et al., 2012), and an increase in human-wildlife encounters (Soulsbury & White, 2015), all of which can have strong impacts on population persistence. Urban areas are now often approached as a habitat type themselves and their impacts are often assessed through a suite of variables that may include the extent of impervious surfaces, roads, housing units, or human population within a defined area. Assessing differing environmental conditions along a gradient of urbanization is a particularly useful tool that can inform about the range of responses possible from organisms inhabiting a variety of human-influenced areas. In the modern day, it is crucial for us to not only understand how urbanized habitats pose risks to wildlife populations, but also how to better manage and mitigate those risks, as cities continue to encroach upon and replace natural habitats.

In addition, climate change has profound effects on varied aspects of organismal biology. Changes in local climate can affect wildlife in multiple ways such as changes in the timing of reproduction, migration, or hibernation; range shifts; or impacts to a variety of other behaviors and physiological processes (Bellard et al., 2012; Inouye, 2022). Climate change not only causes changes in local temperatures, but can alter weather patterns as well, potentially causing additional impacts to local flora and fauna, such as alterations in movement patterns and reproductive timing.

Organisms can respond to anthropogenic change in a number of ways. Some responses may be adaptive while others may be detrimental. While in many cases, wildlife populations cannot survive in the face of rapid anthropogenic changes, in some circumstances, organisms may be able to persist in highly impacted areas. Organisms may respond through phenotypic plasticity, rapid evolution of traits (C. R. Brown & Bomberger Brown, 2013a), range shifts, or a combination thereof. Some species may be rapidly wiped out due to habitat destruction, while others may decline through slower processes that lead to a decrease in effective population size, or in other words, the number of individuals that may contribute genetically to the next generation. Responses to anthropogenic change may be morphological or physiological in nature, or organisms may respond through behavior. While human impacts on many species are negative, some may benefit from factors such as increased temperature (Voigt et al., 2016), the exclusion of predators (Eötvös et al., 2018; Prange et al., 2004; Vincze et al., 2017), a decrease in competitors (Shochat et al., 2010), or infrastructure that mimics natural habitat needs (Voigt et al., 2016).

In addition to the varied ways in which organisms can respond to anthropogenic changes, the nature and severity of anthropogenic impacts may differ across ontogeny. Many studies documenting anthropogenic effects on wildlife are short-term and only consider a single stage of life or a single population. In order to more accurately assess the status of a species, it is necessary to consider how organisms may be impacted differently among life stages, both at the level of the individual and the population. Conditions experienced during the embryo stage have been shown to have effects far-reaching into adulthood (While et al., 2018), and juvenile survival can greatly impact recruitment into a population (Congdon et al., 1993). However, loss of adult individuals in a population can be the most costly for many taxa (Congdon et al., 1993). Differential rates of survival or mortality across life stages or between the sexes can cause changes at the population level, and skewed sex ratios or age ratios can substantially affect a population's ability to persist. These realities highlight the importance of addressing human impacts on wildlife at multiple scales and across different stages of life.

There are several aspects of the life history and biology of turtles that make them especially prone to a variety of anthropogenic impacts. As long-lived animals that experience high levels of juvenile mortality, impacts on adult survival have the greatest influence on population persistence (Congdon et al., 1993). As slow-moving animals that depend on traveling between aquatic and terrestrial habitats, freshwater turtles in urban areas are frequently exposed to altered habitats and urban structures such as roadways, as well as increased interactions with humans. Altered habitat characteristics, as well as disturbance by humans can alter normal behaviors including nesting activity (Moore & Seigel, 2006; Selman et al., 2013). In particular, road mortality can decrease population sizes, disproportionately affect females, and ultimately skew sex ratios (Gibbs & Steen, 2005; Steen & Gibbs, 2004). As oviparous ectotherms, many of their physiological functions are greatly dependent on temperature, and the thermal environment experienced during development can be particularly influential. Incubation temperature has been shown to affect a wide range of phenotypes in reptiles ranging from body size to behavior (Noble et al., 2018; While et al., 2018), meaning alterations to climate that impact nest temperatures can be detrimental. Furthermore, as a group in which many species display temperature-dependent sex determination, changes in climate can also pose risks to the primary and secondary sex ratios of turtle populations (Roberts et al., 2023). The developmentally plastic traits of turtle embryos also brings to light the importance of female nest-site choice, which may be influenced by habitat alterations.

Turtles are among the most threatened groups of vertebrates, and over 60 percent of the 356 turtle species recognized in 2017 are threatened or extinct (Lovich et al., 2018). In addition to factors such as habitat destruction and climate change that put all herpetofauna at risk, turtles have also faced exploitation due to the pet trade and overharvesting for food (Lovich et al., 2018). Urbanization studies on herpetofauna are lacking when compared to other taxa (Collins et al., 2021), and many conservation measures have focused disproportionately on birds, mammals, and amphibians, with few considering reptiles and especially turtles, despite their greater need for conservation action globally (Buhlmann et al., 2009; Lovich et al., 2018; Roll et al., 2017). In addition to their intrinsic value, turtles are important to ecosystem function as a whole, largely due to their high biomass and contributions to the food web both as predators and herbivores, and as prey (Lovich et al., 2018). The southeastern United States and Alabama in particular is a global hotspot for turtle diversity, and has been designated as one of three Turtle Priority Areas for conservation worldwide (Buhlmann et al., 2009).

In my dissertation, I aim to assess human impacts on freshwater turtle populations in Lee, Macon, and Chambers counties, Alabama at different life stages and scales. My first chapter investigates variation in nest-site choice across a local gradient of urbanization. Human impacts on nest-site choice are important to understand because the site a female chooses to place her nest has important fitness consequences, and is influenced by available habitat that may be altered in human-disturbed landscapes (Borges & Marini, 2010; James Reynolds et al., 2019; Kolbe & Janzen, 2002; Ogden, 1991; Refsnider & Janzen, 2010; Xu et al., 2020). My second chapter addresses the effects of incubation temperature and overwintering environment, both potentially altered by humans, on locomotor performance of hatchling turtles. Understanding factors that influence juvenile survival in turtles is crucial, as variation in this life stage may contribute greatly to local population maintenance and growth (Casale et al., 2015; Heppell, 1998). My third chapter deals with variation in sex ratios across a local gradient of urbanization. It is crucial to gain an understanding of whether male and female turtles are differentially affected by urbanization, which could result in decreases in effective population sizes, and ultimately species loss.

Study species

The pond slider, *Trachemys scripta* (Schoepff, 1792; Emydidae) is a freshwater turtle with a native distribution that spans eastern North America (Ernst & Lovich, 2009), two subspecies of which, the red-eared slider and yellow-bellied slider (*T s. elegans and T. s. scripta*), occur in Alabama (Guyer et al., 2015). Pond sliders occupy a variety of habitats with still or slow-moving water and can be found abundantly in both natural and human-made ponds. In Alabama, sliders are typically the most abundant species of turtle encountered. Pond sliders nest from April to August, in open sunny areas, generally within 100 m of water (Ernst & Lovich, 2009; Guyer et al., 2015). Females build a nest cavity up to 11.4 cm deep and lay up to three clutches per year of 4-20 eggs (Ernst & Lovich, 2009; Guyer et al., 2015; Tucker, 2001). Following hatching in late summer or fall, hatchling sliders may either emerge, or overwinter in the nest cavity and emerge the following spring (occurring in over 90% of nests in some accounts) (Gibbons, 2013). Male pond sliders reach sexual maturity between 4 and 6 years of age, while females become sexually mature between 6 and 8 years (Gibbons, 1990).

It is often not feasible to directly assess risks to declining or uncommon species, as their low abundances preclude gathering enough data. Therefore, as a species that is abundant across a natural to suburban gradient in my study area, the pond slider makes an ideal model to study how humans impact freshwater turtles. Although pond sliders are not a species of conservation concern themselves, turtles are at risk as a group, and the findings in my dissertation can likely be applied to other pond turtle species that are at more immediate risk.

Chapter 1: Consistent Nest Site Selection by Turtles across Habitats with Varying Levels of Human Disturbance

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Urbanization dramatically changes the natural landscape and has numerous consequences on local climate, habitat structure, and biota. The damaging effects of urbanization on habitat quality and biodiversity are well documented (Czech et al., 2000; Mcdonald et al., 2008; McKinney, 2002; Pauchard et al., 2006) and can rapidly alter the behavior and survival of native species. A wide range of organismal responses to urbanization have been documented (Alberti et al., 2017; Isaksson, 2015), including rapid acclimation, habituation, and even adaptation to human presence and human-modified environments (Szulkin et al., 2020). For example, many mammal species have acclimated to urbanized habitats by shifting their behaviors, activity budgets and diet preferences (Ritzel & Gallo, 2020). Documenting these types of organismal responses to increasing urbanization is necessary to conserve wildlife populations and to determine ways to maintain key ecosystem functions (Alberti et al., 2017).

Oviparous organisms typically rely on specific habitat characteristics for successful nesting. In most human-disturbed landscapes, however, habitat variables that are important for nest success (e.g., ground substrate, shade cover, temperature, predator densities) are heavily modified (Buxton et al., 2018; Hope et al., 2022; Kolbe & Janzen, 2002). For example, urbanization can generate deviations from optimal thermal and hydric conditions in nests, leading to reduced offspring survival (Angilletta Jr. et al., 2000; Bodensteiner et al., 2015; Christian et al., 1986; Muth, 1980) and skewed population sex ratios for species with temperature-dependent sex determination (suggested by Bowne et al., 2018; N. Jackson et al., 2019). Urbanization can also alter nest-predator (e.g., racoons, dogs, and cats) densities and activity patterns, leading to increased predation rates (Feinberg & Burke, 2003a; Jokimäki & Huhta, 2000; Marchand & Litvaitis, 2004; Wilcove, 1985; but see Foley et al., 2012). Conversely, predator activity or movement may decrease or remain concentrated in pockets of urbanized areas in ways that may reduce rates of nest predation (Eötvös et al., 2018; Prange et al., 2004; Vincze et al., 2017). The environmental effects described above imply that nest site choice is under selective pressure, because it links female behavioral traits to the survival, hence fitness, of her offspring (G. P. Brown & Shine, 2004; D. S. Wilson, 1998). Females typically select nest sites with abiotic conditions that enhance egg hatching success and positively affect offspring phenotypes (Pruett et al., 2019; Warner & Andrews, 2002). Females also choose nest environments that minimize the risk of predation either to themselves or their offspring (Madsen & Shine, 1999; Rand & Dugan, 1983; Refsnider & Janzen, 2010; Spencer, 2002; Warner & Shine, 2008). Accordingly, nest site choice encapsulates multiple issues in urban ecology because it (1) has important fitness consequences, (2) is influenced by available habitat and various biotic and abiotic factors, and (3) is altered in human-disturbed habitats (Borges & Marini, 2010; James Reynolds et al., 2019; Kolbe & Janzen, 2002; Ogden, 1991; Xu et al., 2020). Given the dramatic changes in habitat variables in areas with intense human activity, nesting females must either shift their choice of nest microhabitat or seek out pockets of preferred nest microhabitat across a human-disturbed landscape.

Turtles are a globally imperiled taxon (Turtle Conservation Coalition et al., 2011) and are particularly susceptible to rapid environmental changes associated with urbanization due to a variety of factors associated with their life history (e.g., low survival during early life stages, delayed sexual maturity, environmental sex determination) (Congdon et al., 1993). Moreover, predation on turtle nests is common (Congdon et al., 1983) and can potentially hinder recruitment into adult age classes (Schwanz et al., 2010). Threats caused by urbanization also extend to later life stages, as adult turtles are highly vulnerable to predators (Refsnider et al., 2015) and other urban-associated mortality during nesting forays and other overland migrations (e.g., road mortality; Anđelković & Bogdanović, 2022; Vanek & Glowacki, 2019). Thus, the impacts of environmental change due to urbanization on turtles has received considerable research attention (Patrick & Gibbs, 2010; Steen et al., 2006; Steen & Gibbs, 2004; Witherington, 1992). However, relatively few studies of reptiles have examined how varying degrees of human activity and infrastructure affect nesting habitat and its consequences on nest site choice and nest predation rates (N. Jackson et al., 2019; Kolbe & Janzen, 2002; Tiatragul et al., 2020).

In this study, we examined nest microhabitats chosen by female emydid turtles across areas with varying degrees of human disturbance and urbanization in east-central Alabama, USA, which is within a global biodiversity hotspot for turtles (Mittermeier et al., 2015). Emydid turtles are abundant across all our study areas, which vary in both the degree of human disturbance as well as in available nesting habitat. Given this observation, we hypothesized that females seek and use more specific nest microhabitats (e.g., temperature, shade cover) than what is available across the landscape. This aspect of our study was designed to determine whether turtles discriminate among abiotic factors when selecting a nest site. We also predicted that the level of human disturbance at our study areas would influence maternally selected microhabitat variables. Additionally, we quantified nest predation to provide insight into the role of this biotic factor in shaping nest success across natural to human-disturbed areas. Specifically, we performed an experiment with artificially constructed nests to determine if nest predation rates differ among our study areas with respect to the level of human disturbance. Consistent with our observations, we predicted that rates of nest predation would be highest in areas with less human disturbance.

Methods

Field Data Collection

We located emydid turtle nests surrounding 13 ponds in Lee and Macon counties, Alabama, from May to July 2019 (Table 1.1). Although our study areas contained nearby forested areas, we focused our surveys in open habitat at each area because numerous studies show that emydid turtles choose open habitat for nesting (Najbar & Szuszkiewicz, 2007; Pruett et al., 2019; Roosenburg, 1996). We visited all study areas regularly during the nesting season to maximize discovery of recent nests. We did not formally quantify effort by study area. However, we searched for nests on average six, thirteen, and three times per month for the high, intermediate, and low disturbance study areas, respectively. The difference in visitation rate to the different study area types is due to differences in the number and size of potential nesting areas: high disturbance study areas total approximately 20.3 ha across nine locations, intermediate areas total 1.3 ha across two locations. We found intact nests by observation of actively nesting females or by visual inspection of the ground for nests. Depredated nests, which were visually obvious as partially excavated cavities with eggshells scattered nearby, were also recorded during our survey. Depredated nests could have been from *Trachemys scripta*, *Pseudemys concinna*, *Chrysemys picta*, or *Terrapene carolina*. However, all nests for which the species could be confirmed were of *T. scripta*, the most common emydid turtle in our study areas. Here, we only report data from nests with obviously elliptical eggs characteristic of emydid turtles, as opposed to the spherical eggs of Chelydridae and Trionychidae.

Table 1.1. Study areas where turtle nesting was observed and where the nest predation experiments were performed. The coordinates for the pond on private property were omitted to maintain landowner privacy.

Disturbance Level	**Study Area**	**Pond size (m^2)**	**Coordinates**	**Nesting Study vs. Nest Predation Experiment**
High	Town Creek Park	4561	32.582539, -85.476735	Both
High	Kiesel Park	742	32.587040, -85.542433	Nesting study
High	Longleaf Villas	3131	32.570633, -85.506619	Nesting study
High	Agricultural Heritage Park	8907	32.594622, -85.675574	Predation experiment
Intermediate	Fisheries pond S10	11,558	32.669121, -85.508862	Both
Intermediate	Fisheries pond S11	11,485	32.671127, -85.507211	Both
Intermediate	Fisheries pond S2	7224	32.683346, -85.516154	Nesting study
Intermediate	Fisheries pond S23	5600	32.678296, -85.517820	Nesting study
Intermediate	Fisheries pond S24	7085	32.680441, -85.518099	Nesting study
Intermediate	Fisheries pond S29	11,716	32.669498, -85.501004	Nesting study

Disturbance Level	**Study Area**	**Pond size (m^2)**	**Coordinates**	**Nesting Study vs. Nest Predation Experiment**
Intermediate	Fisheries pond S30	38,286	32.674933, -85.495792	Nesting study
Intermediate	Fisheries pond S8 east	5598	32.672734, -85.507651	Nesting study
Intermediate	Fisheries pond S8 west	37,512	32.672084, -85.509432	Nesting study
Low	Tuskegee National Forest oxbow pond	7342	32.439472, -85.635536	Both
Low	Notasulga pond	11,899	--	Predation experiment

Human disturbance and proximity to human infrastructure varied among our study areas, and as such, we ranked them as having high, intermediate, or low human disturbance (Table 1.1); we later confirmed these rankings with quantitative data on human census population size, amount of impervious surface, road density, and other variables (see details below). High disturbance areas were located at several city parks, as well as an apartment complex, in suburban areas of Auburn, Alabama. These areas were characterized as having infrastructure such as boardwalks and sidewalks adjacent to ponds and frequent human-related activity (e.g., pedestrians and pet dogs often swimming in ponds). Intermediate areas were located at Auburn University's EW Shell Fisheries Center in Auburn, Alabama, which has many ponds located in large grassy fields. These ponds experience periodic management such as grass mowing around the perimeters, but overall infrequent human visitation and little infrastructure. Low disturbance areas were a naturally formed oxbow pond located in Tuskegee National Forest, Macon county, Alabama and a private property pond located in Notasulga, Macon county, Alabama. These areas were far from human infrastructure and experienced very little, if any, human traffic. All study areas were in relatively close proximity to each other (within 30 km) and therefore eliminated the potential for confounding effects of geographic or climatic variation.

We measured several microhabitat characteristics at each intact and depredated nest. We measured the distance between each nest and the water using a measuring tape or wheel, in a straight line to the nearest shoreline of the closest body of water. We measured canopy openness by taking hemispherical photographs above the nest. We used a Nikon Coolpix L30 with a magnetic Zykkor fish eye 0.2X 180 degree lens. Before each photo, we oriented the camera facing north and then set the camera facing lens up directly on top of the nest. We analyzed the photographs with GapLight Analyzer software to obtain percent canopy openness values (Doody et al., 2006). We measured the slope of the ground surrounding the nest using the Apple Measure application.

To compare nest microhabitat to that available across the general landscape, we measured the same microhabitat variables described above at randomly selected artificial nest sites around each natural nest. We identified artificial nest site locations using a random number generator to obtain values between 1 and 10, indicating distance in meters from the natural nest, and values between 0 and 360, indicating the cardinal direction from the natural nest. Three artificial nests were created per natural nest. Henceforth, we refer to maternally selected nests as “natural nests” and randomly selected sites as “artificial nests”.

We placed an iButton temperature logger inside nest cavities (for natural nests and a subset of artificial nests; n = 47 total) for the duration of the nesting season. Early in the season, we placed an iButton in every artificial nest. Later in the season, we placed an iButton in one of every three artificial nests, due to time limitations. iButtons were buried at the approximate depth of the cavity of the natural nest and at the same depth for the associated artificial nests. Temperature was recorded hourly to the nearest 0.5 °C.We trimmed iButton data to a total of 67 days (the average incubation length for T. scripta eggs at 28 °C; unpublished data), starting on the day after the iButton was deployed. Average daily maximum, mean, and minimum temperatures were obtained from all iButtons. We then averaged these values across all 67 days of data to obtain a single average daily maximum, mean, and minimum temperature value for each natural and artificial nest. Additionally, the average daily temperature range value for each iButton (maximum–minimum) was calculated. Each of these temperature variables was then used as a microhabitat variable in our analyses.

Nest Predation Experiment

We conducted an experiment to assess variation in nest predation across different levels of human disturbance at our study areas. Our experiment was performed at ponds of similar size at two areas with high human disturbance, two with intermediate disturbance,

and two with low disturbance (Table 1.1).

We measured the perimeter of each pond that contained typical nesting habitat (open, grassy), placed a flag at each 10m section, and assigned each section a number. Five sections (marked with flags) were selected using a random number generator; an artificial nest was constructed at each section at 3 m from the edge of the pond. Artificial nests had a cavity similar to that of a natural nest and consisted of a chicken egg buried in the soil at 10 cm depth (comparable to the depth of an emydid turtle nest). Past studies have successfully used bird eggs to quantify rates of predation on turtle nests (Dawson et al., 2014; Foley et al., 2012; Marchand et al., 2002; Marchand & Litvaitis, 2004; Ratnaswamy et al., 1997; Wilhoft et al., 1979). We moistened the disturbed soil with pond water to simulate when female turtles release water from their bladder before nesting. Because nest predators rely on olfactory and visual cues to locate turtle nests (Strickland et al., 2010), we reasoned that disturbed soil and the pond water provided realistic cues that simulated those of natural nests (Buzuleciu et al., 2016). To discreetly mark the location of each artificial nest, we removed the flags and placed two wooden dowels one meter away from each nest. After burying eggs, we checked each artificial nest once every 24–48 h for a total of 72 h (as most predation occurs within this timeframe, Congdon et al., 1983, 1987; Holcomb & Carr, 2013); but see Riley & Litzgus, 2014). On each visit, evidence of predation, such as an excavated cavity or the presence of eggshells, was recorded and remaining eggs were removed after 72 h. This process was performed 3 times at each location, totaling 90 eggs buried across all six study areas. We randomly selected different sections of the pond for each repetition and waited at least 48 h before burying a new set of eggs at each section.

Quantifying Urbanization

To quantify the level of human disturbance at each study area, we downloaded data from the United States Environmental Protection Agency StreamCat Dataset that contains standardized measures of natural and anthropogenic features of streams, their catchments, and watersheds (Hill et al., 2016). For each pond where we measured nest microhabitat or conducted the predation experiment, we used the WATERS GeoViewer (https: //www.epa.gov/waterdata/waters-geoviewer (accessed on 20 September 2022)) to select the closest stream segment to the ponds at our study areas and downloaded the StreamCat data associated with the catchment area draining into that segment. Five variables were extracted from the watershed dataset: mean imperviousness of anthropogenic surfaces within catchment, percentage of local catchment area classified as developed, average density of roads per square kilometer, mean of all housing units per square kilometer, and mean of 2010 census population per square kilometer. For ponds that were equidistant to two segments, we took the average of the two segments for each variable. We performed a principal components analysis (PCA) on these five variables across each of our study areas. The first principal component (PC1) explained 88.62% of the variation in the data and was used in our subsequent analyses as a continuous variable associated with the level of human disturbance. We multiplied PC1 scores by negative one to facilitate ease of interpretation (Table 1.2) so that positive PC1 scores indicated high human disturbance, while negative scores were indicative of more natural areas with low human disturbance. Importantly, because our original classification of study areas as “high disturbance” vs. “intermediate” vs. “low disturbance” were reflected in the results of the PCA (Figure 1.1), some of our analyses also used the study areas as their original designations (rather than use PC scores). Although our sample of nests was low in the high disturbance study area (see Results), we still distinguished this study area type from the others due to dramatically different surroundings, infrastructure, and levels of human activity. We will refer to the original designations as “study area type” and to PC1 as “urbanization level”.

Table 1.2. Loadings and proportion of variance explained on different principal component (PC) axes from a Principal Components Analysis.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Impervious surface	0.4624	0.0363	-0.6312	-0.6137	-0.0988
Developed area within catchment	0.4732	0.0675	-0.0300	-0.2563	0.8396
Density of roads	0.4045	0.7692	0.4165	0.0355	-0.2641
Housing units	0.4270	-0.5903	0.6012	0.3196	-0.0742
Human population	0.4649	-0.2319	-0.2562	-0.6740	-0.4583
Proportion of variance explained	0.8862	0.08643	0.0215	0.0046	0.0011

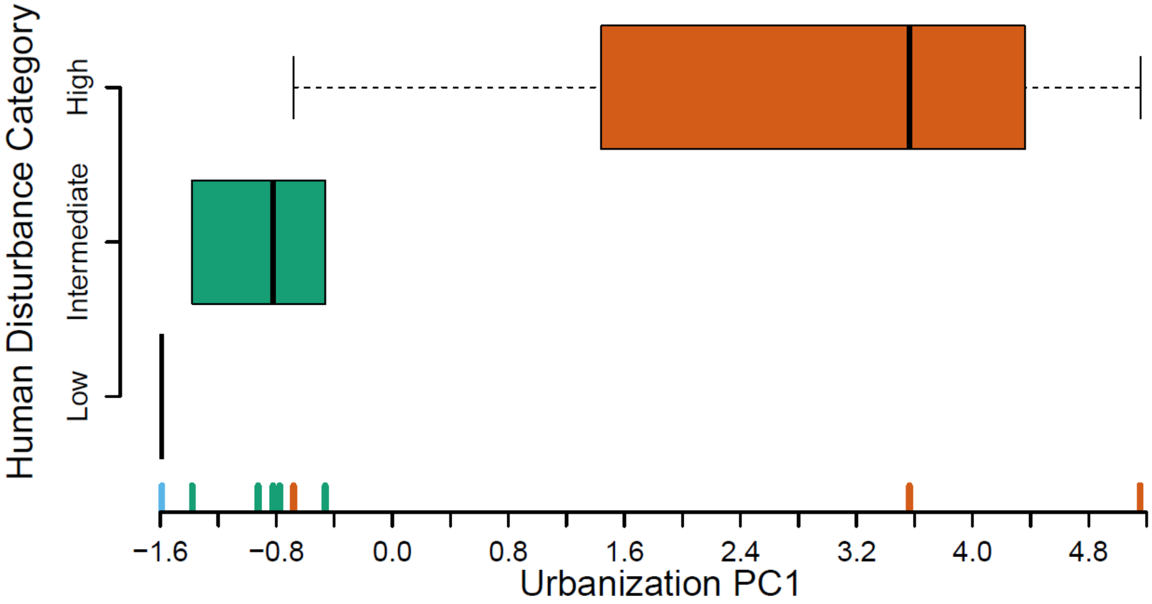

Figure 1.1. Level of human disturbance across study areas. Initial designation of human disturbance included three categories based on our observations (high, intermediate, low levels of disturbance). Principal Component Axis 1 (PC1) combines different metrics of human disturbance across study areas (see Table 1.2). Colored ticks along the x-axis indicate actual PC1 scores of each study area (some study areas within a human disturbance category have the same PC1 score).

Statistical Analysis

To broadly characterize high, intermediate, and low human disturbance study area microhabitats without respect to turtle nest site choice, we built linear models with a dataset that included artificial nests only, as these randomly located sites should provide a general description of the habitat at each study area. We used six models, each with a microhabitat variable (listed in Table 1.3) as the dependent variable, and study area type as a categorical fixed effect. We also included iButton depth as a continuous covariate in models that included a temperature-dependent variable. Distance from water was not included in this analysis since this variable is only meaningful in comparison with natural nests.

Table 1.3. Test statistics for comparisons of randomly selected locations (i.e., artificial nests) across three different types of study areas (high, intermediate, and low levels of human disturbance). Comparisons of temperature variables included iButton depth as an additional independent variable. For comparisons among study area types, effect sizes (β) were calculated using the low human disturbance area as the reference. Standard errors are indicated in parentheses, and statistically significant p-values are in bold font.

Dependent variables	**Study area type**	**iButton depth**
Slope	$F_{2,124}$ = 5.792, **p = 0.004**	-
Intermediate disturbance	β = 5.106 (1.602), **p = 0.002**	
High disturbance	β = 6.233 (2.707), **p = 0.023**	
Canopy openness (%)	$F_{2,126}$ = 4.573, **p = 0.012**	-
Intermediate disturbance	β = 7.852 (4.238), p = 0.066	
High disturbance	β = -10.898 (7.197), p = 0.133	
Average daily mean temperature	$F_{2,19}$ = 1.946, p = 0.170	β = -1.778 (1.246)

Dependent variables	**Study area type**	**iButton depth**
Intermediate disturbance High disturbance	$\beta = -1.005$ (1.168), $p = 0.400$ $\beta = 1.179$ (2.272), $p = 0.610$	$p = 0.170$
Average daily maximum temperature Intermediate disturbance High disturbance	$F_{2,19} = 3.565$, **$p = 0.048$** $\beta = -2.627$ (2.076), $p = 0.221$ $\beta = 2.263$ (4.039), $p = 0.582$	$\beta = -3.877$ (2.214) $p = 0.096$
Average daily minimum temperature Intermediate disturbance High disturbance	$F_{2,19} = 0.131$, $p = 0.878$ $\beta = -0.036$ (0.645), $p = 0.956$ $\beta = 0.588$ (1.256), $p = 0.645$	$\beta = -0.417$ (0.688) $p = 0.552$
Average daily temperature range Intermediate disturbance High disturbance	$F_{2,19} = 5.587$, **$p = 0.012$** $\beta = -2.591$ (1.573), $p = 0.116$ $\beta = 1.675$ (3.061), $p = 0.590$	$\beta = -3.460$ (1.678) $p = 0.053$

To determine if females discriminate among abiotic factors when selecting nest sites, and whether microhabitat measures varied with level of disturbance, we included a set of seven linear mixed-effects models, each with a microhabitat variable as the dependent variable. We included nest type (natural vs. artificial nest) as a categorical fixed effect, urbanization level (PC1 described above) as a continuous covariate, and the interaction between those two variables. For models of temperature-dependent variables, a continuous covariate of iButton depth (mean centered and standard deviation scaled) was also included. Statistical significance of individual fixed effect terms was evaluated with an analysis of variance implementing incremental sums of squares. Nest cluster (a natural nest and its associated artificial nests) was assigned as a random effect in each model to account for the non-independence of nests within clusters. To determine whether female turtles selected nest sites with more or less variance in microhabitat measures, we estimated separate residual variances for natural and artificial nests. We used the asreml package (Butler et al., 2009) in R for each model and obtained 95% confidence intervals for the residual variances using profile likelihoods (Meyer, 2008) implemented in the nadiv package (Wolak, 2012). A likelihood ratio test was used to evaluate the statistical null hypothesis that there is no difference in residual variance between natural and artificial nests.

To determine if nest predation rate was associated with study area type, we performed a Pearson's Chi-square test for independence, using the numbers of artificial nests that were depredated and the number that survived at each of the three study area types (high, intermediate, low disturbance). All statistical analyses were performed in R, version 4.2.1 (R Core Team, 2022).

Results

Slope of the ground and canopy openness measured at artificial nests (i.e., random locations representative of the landscape at each study area) varied with the level of human disturbance (Table 1.3). Ponds in more human-disturbed areas had steeper terrain and less canopy openness than those in natural areas. Mean and minimum ground temperatures did not change substantially with the level of human disturbance, but the daily maximum and daily range of ground temperature decreased with increasing human disturbance (Table 1.3).

Nest Site Choice

A total of 88 nests (11 intact, 77 depredated) were located during the study. Most nests (n = 84) were at intermediate or low disturbance areas, and only four were found in high disturbance areas. Due to logistical limitations, microhabitat data were collected on a subset of natural nests and their associated artificial nests (n = 43; 4 high, 25 intermediate, 14 low), and all results reported are from this set of nests. In addition, due to failure or loss of some iButtons, temperature data were available for 20 natural nests and 27 artificial nests.

Microhabitat variables measured at natural nests generally followed the same trends as those in artificial nests (Figure 1.2). Slope, daily mean, and daily maximum temperature were marginally significantly related to the level of urbanization (Table 1.4), and these relationships did not differ between natural and artificial nests. Nests in more urban areas were on steeper slopes than in natural areas (Figure 1.2B). Average nest temperature at natural areas (29.9 °C, n = 11) was about 2 °C warmer than that at intermediate areas (28.0 °C, n = 8), and daily maximum temperatures followed a similar trend; higher maxima in natural areas as compared to intermediate and high human disturbance locations (Figure 1.2). Although temperature data from only one natural nest iButton were available at a high human disturbance area, this trend was still observed when we compared just the intermediate to natural areas. The distance of natural nests to the nearest pond ranged from 2 to 241 m, with 88% of nests being within 50 m of a pond; this pattern did not vary with the level of human disturbance.

Although nest site microhabitat characteristics did not differ on average between natural and artificial nests, natural nest sites generally exhibited less variance compared to artificial nests. For instance, there was significantly ($p < 0.05$) less among-nest variance of natural nests in their distance to water, canopy openness, and average daily mean and minimum temperatures. However, the variance of daily temperature range of natural nests was greater than that of artificial nests. For other microhabitat measures, the best fit model indicated that residual variance was equal between natural and artificial nests.

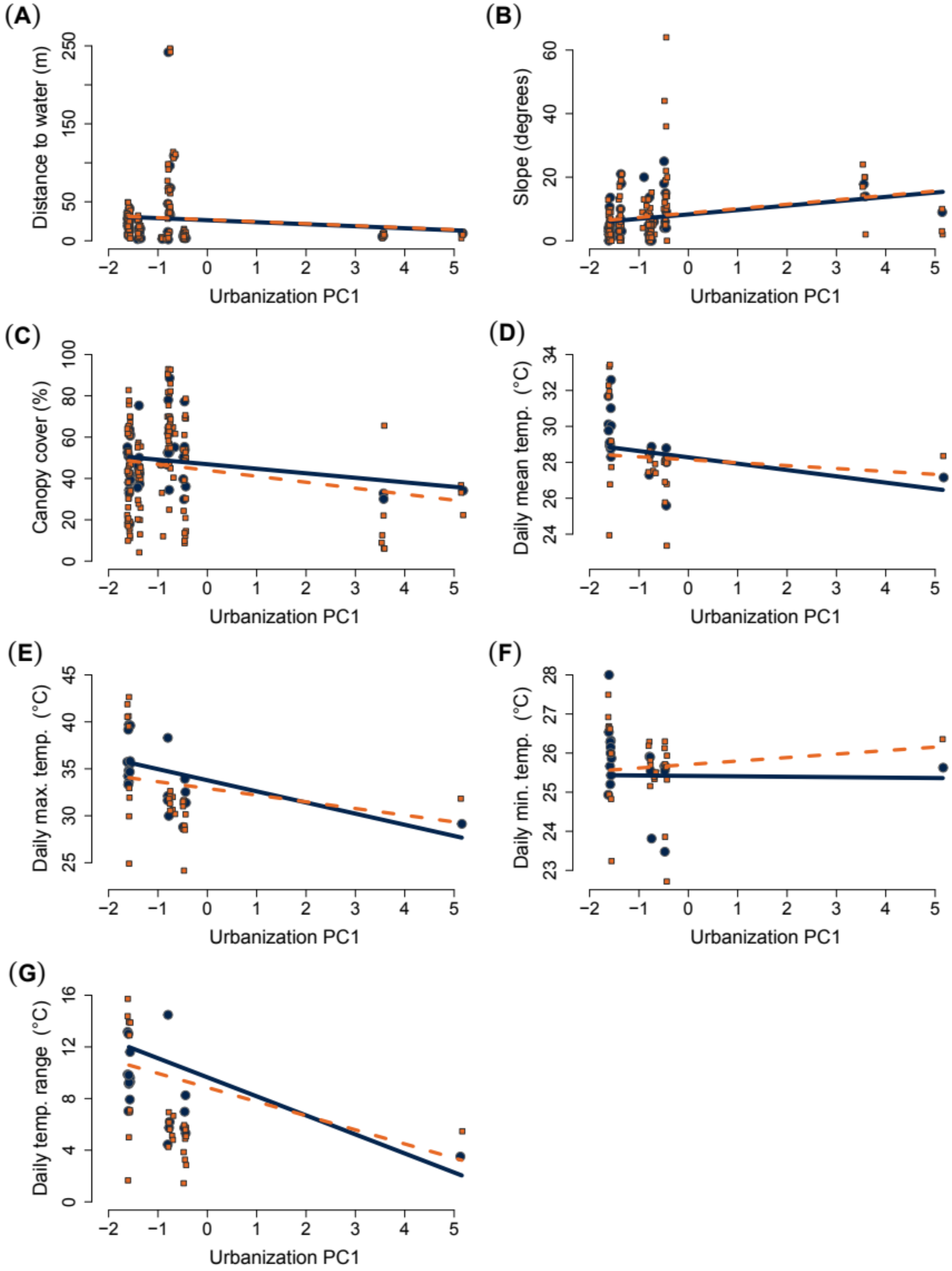

Figure 1.2. Effects of urbanization level for natural (blue circles, solid lines) and artificial (orange squares, dashed lines) nests on microhabitat variables. A) Distance of nests from the edge of water of the nearest pond. B) Ground slope around nest sites. C) Canopy cover over nests. D) Daily mean nest temperature. E) Daily maximum nest temperature. F) Daily minimum nest temperature. G) Daily temperature range.

Table 1.4. Effects of urbanization, nest type (natural vs artificial), and their interaction on microhabitat variables. iButton depth was only included in the analyses of temperature data. The effect size for nest type was calculated with the natural nest as the intercept/reference. The rightmost two columns indicate whether residual variance differed between natural (N) and artificial (A) nests, and if so, what the two separate variances are (natural nests listed first, artificial nests second). Estimates and either standard errors or 95% confidence intervals are indicated in parentheses. For residual variances, we report lower confidence interval limits of 0 when the CIs cannot exclude zero. Statistically significant p-values are in bold font.

	Urbaniz. level (PC1)	Nest type	Urbaniz. level x nest type interaction	iButton depth	Residual variances	Equal variance likelihood ratio test
Distance from water	β=-2.589 (4.504) p=0.567	β=0.625 (0.523) p=0.186	β=0.020 (0.328) p=0.951	-	N=4.875 (2.331 to 8.453) A=12.889 (10.507 to 16.048)	λ_1=5.025 **p=0.012**
Slope	β=1.377 (0.831) **p=0.058**	β=0.428 (0.898) p=0.594	β=0.006 (0.562) p=0.991	-	N=19.166 (12.147 to 29.477) A=23.457 (18.740 to 29.871)	λ_1=0.263 p=0.304
% canopy openness	β=-2.189 (1.826) p=0.124	β=-2.931 (2.259) p=0.228	β=-0.704 (1.415) p=0.619	-	N=90.671 (49.070 to 153.127) A=241.750 (191.827 to 306.462)	λ_1=4.712 **p=0.015**
Average daily mean temp.	β=-0.339 (0.372) p=0.095	β=-0.134 (0.409) p=0.522	β=0.189 (0.262) p=0.470	β=-0.021 (0.707) p=0.973	N=0.029 (0 to 1.228) A=2.659 (1.530 to 4.302)	λ_1=2.781 **p=0.048**
Average daily	β=-0.333 (0.819) p=0.077	β=-0.871 (0.962) p=0.150	β=0.472 (0.571) p=0.408	β=-1.435 (1.1494) p=0.335	N=3.777 (0 to 20.031) A=8.985	λ_1=0.321 p=0.286

	Urbaniz. level (PC1)	**Nest type**	**Urbaniz. level x nest type interaction**	**iButton depth**	**Residual variances**	**Equal variance likelihood ratio test**
max. temp.					(1.899 to 17.140)	
Average daily min. temp.	β=0.032 (0.232) p=0.994	β=0.291 (0.183) p=0.174	β=0.100 (0.115) p=0.385	β=-0.072 (0.437) p=0.865	N=0.008 (0 to 0.279) A=0.512 (0.317 to 0.835)	λ_1=3.064 **p=0.040**
Average daily temp. range	β=0.482 (1.002) p=0.222	β=-0.793 (1.051) p=0.241	β=0.382 (0.578) p=0.508	β=-3.296 (1.605) **p=0.040**	N=11.348 (3.224 to 23.909) A=1.751 (0.828 to 7.223)	λ_1=1.205 p=0.136

Nest Predation Experiment

Of the 90 artificial nests, we documented six instances of nest predation, and nest predation rate was significantly greater in natural areas than the intermediate and high disturbance areas (chi-square = 7.5, p = 0.045). Five of the six nests depredated were at natural areas (17% predation rate), and the remaining was at an intermediate disturbance area; no predation occurred in high disturbance areas. Out of the five nests depredated in natural areas, four occurred at the oxbow pond in Tuskegee National Forest, while one occurred at the private property pond in Notasulga, AL. Similarly, during our nest surveys, more depredated nests were observed in low (n = 27) and intermediate (n = 49) human disturbance study areas than at high disturbance areas (n = 1).

Discussion

Human activity has altered natural landscapes in ways that have dramatic effects on wildlife populations. These effects of habitat alteration may be particularly pronounced when they directly impact habitat or other environmental factors that are important for reproduction (Reale & Blair, 2005; Sumasgutner et al., 2014), such as nesting areas of oviparous species. In this study, we quantified variation in nesting habitat for turtles, maternal nesting behaviors, and nest predation rates across a range of areas that vary in the level of human disturbance. We found that nesting landscapes in areas with greater human disturbance had steeper slopes and reduced canopy openness than in areas with less human disturbance. The reduced variance in some microhabitat variables for natural nests suggests that female turtles choose microhabitats with specific abiotic conditions, and these patterns remained consistent across the study. Nest predation also varied among study locations and was substantially lower in areas with high levels of human disturbance. These patterns illustrate that while human activities alter natural habitats, female turtles are still capable of finding nest sites with similar microhabitats across different levels of disturbance.

While the distance of nests to water and average canopy over nests tended to reflect what was available across study areas, female turtles nested in a narrower range of these variables than what was measured at artificial nest sites, suggesting that females are selective for distance from the water's edge and canopy when choosing a nest site. However, we detected minimal differences for maternally selected nest microhabitat characteristics among different study area types on an urban gradient, suggesting that females discriminate among abiotic factors when selecting nest sites but generally select sites with similar characteristics at each level of human disturbance. Nesting turtles may not adjust their nest site choice when faced with anthropogenic change, highlighting the need for protecting areas with suitable habitat for nesting and egg incubation, particularly areas that are altered by humans. However, more investigation is needed to determine whether turtles are capable of adjusting their nesting strategy in these areas or whether they were able to find appropriate nest sites without substantial changes to their nesting behaviors.

Nest temperatures were relatively high in natural areas compared to high and intermediate disturbance areas. This result contrasts with those of studies on the urban heat island effect whereby urbanized areas are considerably warmer than surrounding natural areas due to a variety of factors (e.g., increased heat absorbing surface, decreased tree cover; (Arnfield, 2003)). Notably, our study areas with the highest levels of human disturbance were more suburban than urban and still contained substantial amounts of greenspace (i.e., city parks) that would reduce the likelihood of elevated temperatures comparable to those in large cities. The range of human disturbance in our study does not extend to the extremes seen in large cities (Peng et al., 2012). Consequently, variation in temperature across our study areas is more subtle, especially with the limited temperature data collected for nests in our most human-disturbed areas. Nevertheless, we detected increased nest temperatures in natural areas, which could be driven by substrate differences; all nests at the natural location had pebbly substrate that may absorb more heat than the grassy/soil substrate at our more urban study areas. These results highlight the importance of considering local microhabitat characteristics in driving thermal patterns across urban to natural gradients, as major assumptions in urban ecology (e.g., urban areas are always relatively warm; Arnfield, 2003) may not always be met. Relying solely on these broad assumptions, especially when the gradient is relatively shallow, could impact the success of management efforts for wildlife species.

The artificial ponds at our high and intermediate human disturbance areas were surrounded by steeper terrain than ponds in natural areas, which is a common characteristic of human-made wetlands that were carved out by construction equipment. Consequently, turtles nested on relatively sloped ground at these areas, which reflects this feature of human-made ponds, rather than being indicative of turtle nest site choice. Nevertheless, a high occurrence of steep slopes in nesting areas could affect ambient conditions of the nest that have important consequences on embryo development. For example, sloped banks around the pond will affect radiant heating from the sun (especially on south-facing slopes in the northern hemisphere) and have impacts on nest temperature in ways that influence critical aspects of development (Breitenbach et al., 1984; Noble et al., 2018). Sloped banks could also influence water run-off and decrease the moisture absorbed in the soil at a nest site; these impacts on nest moisture could also influence development of turtle embryos (K. D. Cagle et al., 1993; Packard et al., 1987). Thus, while properly managed artificial wetlands can support healthy wildlife populations (Chovanec, 1994), the surrounding features of artificial ponds are often very different from that of natural ponds and may influence nesting behavior and embryo ecology of turtles in unique ways.

The abundance of nests was relatively low in areas with high human disturbance, despite a high abundance of turtles observed in ponds at these study areas. Over 70 adult turtles can readily be counted (within 1–2 min) in the ponds at city parks and apartment complexes (pers. obs.), whereas few, if any, turtles are observed within this short timeframe at our study areas with low human disturbance; these observations are mostly driven by turtle habituation to humans, as they are often fed by visitors at city parks (as seen in other wildlife; Orams, 2002) and not at the undisturbed areas. Given the high apparent densities of adult turtles in city parks, the lack of nest sites was unexpected, which may be due to several factors. First, females may choose nest sites far outside of our survey areas. While it is possible that females may travel farther distances across land in human-disturbed areas, we argue that this is unlikely because of barriers around the parks (roads, residential areas). Alternatively, females may nest in heavily shaded forest patches surrounding the city parks where we did not search, but a large amount of literature consistently shows that emydid turtles select open habitat for nesting (Kolbe & Janzen, 2002; Pruett et al., 2019; Spencer & Thompson, 2003), which was abundant at our human-disturbed study areas. Second, human disturbance may have caused diel shifts toward nocturnal nesting activity outside of our survey hours. Adaptive shifts toward nocturnal nesting activity at human-disturbed areas is unlikely given the long lifespans of turtles and the relatively short time since establishment of our city park study areas. In addition, other studies provide no evidence that emydid turtles shift the diel timing of nesting activity, as they readily nest during the day in areas with high human activity (Janzen & Morjan, 2001). The lack of depredated nests at city parks also implies little nesting activity outside the times of our surveys. A third explanation involves potential physiological effects of human disturbance that may inhibit reproduction. Trapping efforts (in 2021 and 2022) show that almost no females in city park ponds are gravid during the reproductive season and smaller, juvenile turtles are rarely observed (unpubl. data), indicating low recruitment. Although urbanization has not previously been shown to affect abundance of immature turtles (Bowne et al., 2018), previous work compared populations across a much larger geographic scale than our study. Frequent feeding of low-quality food (i.e., bread) by the public to wildlife can often result in disease and poor nutrition (Murray et al., 2016), which could be responsible for low fecundity, but these potential effects need to be further explored.

Nest predation varied across the study areas with respect to human disturbance. During our surveys, depredated nests were most common in natural and intermediate areas and declined with increasing levels of human disturbance. Although predation rates of simulated nests were low overall, our experimental study lessened concerns about our low detectability of nests in human-disturbed areas and suggests a negative relationship between nest predation and level of human disturbance. This pattern is consistent with studies that suggest that human presence may frighten potential predators (Leighton et al., 2010; Marchand & Litvaitis, 2004), even though human disturbance can sometimes increase populations of nest predators (Thorington & Bowman, 2003). The impact of human disturbance on nest predators can vary considerably and may have variable consequences on rates of nest predation. If female reproduction and nesting activity is low in areas of high human disturbance (e.g., city parks), then mammalian predators may not have had the same opportunity to develop a visual or olfactory search image for turtle nests at these locations. These predators can quickly learn how to locate food items when they become abundant (Dalgish &

Anderson, 1979; Pelech et al., 2010), but if nest abundance remains low at human-disturbed areas, then it is unlikely predators would have been searching for nests during the 72 h period when simulated turtle nests were present during our experiment.

Increased human activity and alteration of landscapes has the potential to substantially change the nesting behavior and ecology of oviparous species (Tiatragul et al., 2020). Turtles are particularly vulnerable to changes in their nesting habitat since biotic and abiotic environments within and around nest sites largely determine egg hatching success (Buhlmann & Coffman, 2001; Packard et al., 1989; Temple, 1987; Tucker & Paukstis, 2000), which in turn could impact population demographics. Understanding how human disturbance impacts nesting sites and changes in maternal nesting behaviors will provide important information for predicting how populations of oviparous animals will persist in human-disrupted environments. We show that maternal nest site choice is relatively consistent across areas with different levels of human disturbance, despite some differences in habitat features across our study areas. The general lack of behavioral shifts in human-disturbed areas suggests that preserving natural microhabitat features will be important for populations in areas with human activity. Moreover, limited evidence of reproduction in areas with high human activity is alarming and warrants more research. Future studies that quantify the effects of human disturbance on offspring development within nests (Lloyd & Warner, 2019; Tiatragul et al., 2020) will provide further insight into how human activities affect recruitment and long-term persistence of populations in anthropogenically modified environments. Overall, our study highlights an important and relatively understudied aspect of reptile biology (i.e., nesting behavior) that warrants more attention in research programs aimed at understanding the impacts of anthropogenic environmental change. As urbanization and human populations continue to increase, it is crucial to document how urbanization impacts turtle nesting behavior, as well as nesting success across systems, species, and types of human disturbance.

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Chapter 2: Incubation and overwintering conditions influence righting performance of hatchling turtles

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Environmental conditions experienced during early life can influence an organism's phenotype (Angilletta, 2009; Arnold, 1983; Booth, 2006; Myers et al., 2007; Shine et al., 1997). These plastic responses to the developmental environment have been documented across taxa (West-Eberhard, 2003; While et al., 2018), and in some cases can generate phenotypes suited to local environments (Reed et al., 2010; Sun et al., 2021) in ways that positively affect fitness (Lind & Johansson, 2007). The timing of exposure to environmental cues can also have large effects on different traits. For example, the egg incubation temperature during the middle third of development determines the sex of the offspring in many reptiles (Yntema, 1979) and diet during the larval stages can determine adult wing coloration in the speckled wood butterfly (*Pararge aegeria*) (Talloen et al., 2004). The importance of the timing of exposure to different abiotic conditions is likely most pronounced in species that have several distinct early life stages that occupy different environments (e.g., holometabolous insects and amphibians). Early life conditions can have fitness consequences at later stages (Cam et al., 2003; Marshall et al., 2017; Mitchell et al., 2013), but the effects of conditions during early development on adult fitness are still poorly studied, especially in long-lived taxa (Mitchell et al., 2018; Refsnider et al., 2019).

Thermal developmental environments are particularly important in ectothermic organisms because most of their physiological functions are greatly dependent on temperature (Angilletta, 2009). For example, temperature can influence a broad range of phenotypic traits (Pottier et al., 2022; Rodrigues & Beldade, 2020; Weeks et al., 2022) as well as the rate of development (Ewert, 1985; Georges et al., 2005), thereby affecting the timing when organisms enter a subsequent stage and their phenotypes at these transitions (Albecker et al., 2023). Oviparous reptiles are particularly prone to environmental effects as their embryos are exposed to the conditions of the oviposition site (Refsnider & Janzen, 2010; While et al., 2018). Egg incubation temperature has consistently been shown to affect developmental rate, embryonic yolk metabolism (Warner et al., 2012), and a range of physiological, morphological, and performance traits of hatchlings (Noble et al., 2018; Refsnider et al., 2019). Incubation-induced phenotypes can have fitness consequences at juvenile and adult stages (Mitchell et al., 2018), and can interact with effects from other developmental stages (Gilbert et al., 2024; Hoffman et al., 2023). Furthermore, offspring of oviparous reptiles that lack parental care are not only subjected to maternally-selected environmental conditions during development, but also at hatching and possibly longer into the juvenile stage (Refsnider & Janzen, 2010; Weisrock & Janzen, 1999), which can have lasting effects into adulthood (Benard & McCauley, 2008).

While embryos of oviparous reptiles cannot behaviorally escape their nest environment, some aspects of maternal nest-site choice may affect important variables that follow incubation, such as the timing of hatching (Riley et al., 2020; Spencer, 2002), the timing of emergence or dispersal from the oviposition site (Gibbons, 2013; Paterson et al., 2014), and habitat choice following dispersal (Paterson et al., 2014). Altogether, these sources of variation in conditions during early life can produce a wide array of phenotypes in juveniles, potentially leading to variation in survival and fitness. The impacts of embryonic conditions on early life are well documented, but we have a poorer understanding of the impacts of post-hatching environments on juvenile and adult success (Benard & McCauley, 2008), and the extent to which individuals can modify their phenotype and mitigate detrimental effects during earlier stages.

The pond slider (*Trachemys scripta* Schoepff, 1792) is a species of freshwater turtle whose native distribution spans across eastern North America (Ernst & Lovich, 2009). Similar to other aquatic turtles, females emerge from wetland habitat to nest on land, where they dig a shallow cavity (maximum depth of 11.4 cm; Ernst and Lovich, 2009) in the ground where eggs are laid, covered, and left to incubate on their own. Females often select thermally variable microhabitats for nesting, and consequently, eggs are exposed to a broad range of temperatures (Du & Shine, 2015; Folkerts Caldwell et al., 2023; Kleewein, 2015). Phenotypic development of offspring is influenced by nest temperature (e.g., offspring size, sex; Noble et al., 2018; While et al., 2018), and eggs produced early in the season that experience warm temperatures will hatch sooner than those produced later in the season or that experienced cool temperatures (Ewert, 1985; Georges et al., 2005).

Embryonic development of pond sliders is followed by a distinct life-history stage where hatchlings typically (but not always) remain inside their nest until the following spring. Following hatching, usually from April through July (Gibbons, 1990), juvenile pond sliders follow one of two strategies for overwintering: 1) overwintering in the nest and emerging in the spring, a behavior performed in possibly over 90% of nests (Gibbons, 2013) or 2) emerging in the fall and overwintering in an aquatic environment.

Selection should favor hatchlings that leave the nest to enter the aquatic environment at the time of year which most benefits their growth and survival (Gibbons, 2013). The environmental or genetic factors that contribute to variation in emergence time of hatchling turtles is not entirely understood, and no species studied in the southeastern United States exclusively uses one strategy over another (Gibbons, 2013). Nonetheless, differing thermal and hydric conditions that fall- versus spring-emerging hatchlings experience during winter, and differing levels of activity are likely to produce variation in survival. For example, aquatically overwintering individuals may spend time active and feeding prior to and during winter, especially in warmer climates (Costanzo et al., 2008; Schubauer & Parmenter, 1981). In contrast, terrestrially overwintering individuals spend several months underground before emerging. These hatchlings must remain in an inactive state in which they are unable to feed, surviving on residual yolk, lipid and carcass reserves, and a reduced metabolism (Costanzo et al., 2008; Muir et al., 2013; Nagle et al., 2000; Warner et al., 2024).

A potential source of variation in hatchling survival is the interaction between developmental rate (driven by egg incubation temperature) and emergence timing. Variation in hatch dates (resulting from different incubation temperatures and oviposition dates) produces variation in the amount of time hatchlings spend in their overwintering environment, whether that be aquatic or terrestrial. This is important for hatchling sliders because they rely on residual yolk stores before emergence (Tucker et al., 1998). Residual yolk is almost completely metabolized before winter in hatchling painted turtles that also overwinter in the nest (Muir et al., 2013; Warner et al., 2024). Warm nest temperatures accelerate development and result in earlier hatching than those experiencing cooler nest temperatures (Ewert, 1985; Georges et al., 2005). These early-hatched individuals will then experience warm summer temperatures that may result in increased metabolism of residual yolk reserves, compared to those that hatch later in the season. Consequently, individuals that hatch early and become active in their aquatic environment will need to satisfy much greater energy demands before winter than those on the opposite end of the spectrum that hatch late and remain in their nest; this could lead to important interactive effects of nest temperature and overwintering environment on offspring performance and survival the following spring.

After emergence, hatchling turtles are particularly vulnerable as they are exposed to a range of abiotic conditions and heightened levels of predation as they travel from the nest to water (Janzen et al., 2000; Wilbur, 1975). The ability to travel from the nest to water is likely influenced by neuromuscular coordination and locomotor performance, which in turn, can influence variation in survival during dispersal. Therefore, hatchling locomotor performance is often considered a fitness-relevant variable. The righting response is a commonly used metric to assess hatchling turtle performance, and involves measuring the time it takes a turtle to return to a right-side-up position after being placed upside-down on its carapace (Delmas et al., 2007). A faster righting time may indicate better physical condition or coordination (Steyermark & Spotila, 2001). The ability of hatchlings to quickly right themselves may also reduce time exposed to predators as well as the risk of desiccation or starvation (Van Casteren et al., 2024). While a direct correlation of the righting response with survival in the field or dispersal ability is rarely supported (Carter et al., 2016; Mitchell et al., 2017), the righting response is repeatable, varies among individuals and families (Carter et al., 2016) (thus, possibly a heritable basis of variation), and is at least indirectly related to survival (Delmas et al., 2007). Because nest temperature during incubation and conditions within nests during winter can generate variation in phenotypes at emergence (Mitchell et al., 2013), the interactive effect of conditions during these two stages may affect survival at the juvenile stage; such effects may shape important aspects of life history and population persistence and growth (Casale et al., 2015; Heppell, 1998). While righting performance does not differ between fall and spring emerging painted turtles (Riley, Tattersall, et al., 2014), direct comparisons after individuals spend the winter in either aquatic or terrestrial habitats have yet to be investigated.

Our aim is to determine the influence of egg incubation temperature and overwintering conditions on morphology and righting ability in hatchling pond sliders, and whether those two stages may have an interactive effect on these traits. Because overwintering in the nest is the predominant condition, we hypothesize that this overwintering location has positive effects on fitness-relevant phenotypes and that incubation temperature can modify the effect of the overwinter environment. We predict that incubation temperature will influence hatchling morphology, and that terrestrially overwintering hatchlings will have faster righting times than aquatically overwintering hatchlings. Furthermore, we predict that incubation temperature during embryonic development will influence hatchling righting times, as well as interact with the effect of the overwintering environment. Pond sliders have been the subject of a variety of studies involving the effects of incubation temperature on sex-determination and other phenotypes (Les et al., 2009; Ligon et al., 2012; Rhen et al., 1999; Treidel et al., 2016; Wibbels et al., 1998). However, no study to date has assessed the effect of the overwinter environment, or its interaction with incubation temperature, on hatchling pond slider locomotor performance.

Materials and Methods

Egg collection and incubation; hatchling housing

From mid May to late July in 2019 and 2020, we collected clutches of pond slider eggs (*Trachemys scripta scripta* and *T. s. elegans*) from nests in Lee County, Alabama, USA. Some clutches of eggs were freshly laid when collected while others were found at an undetermined age. We transported eggs to our laboratory at Auburn University, and measured and weighed them before placing them in incubators. We incubated eggs at five different constant temperatures (24°C, 26°C, 28°C, 30°C, and 32°C). Eggs were placed in groups of six within plastic boxes (20 x 15 x 9 cm) that contained moist vermiculite (-150 kPa) that was rehydrated once a week. Eggs from each clutch were assigned randomly to incubation treatments and locations within boxes. The location of each box within an incubator was also randomized. Hatching took place between July and October. Eggs were checked once per day for hatchlings, and the dates when hatchlings started pipping and when they completely emerged from the egg were recorded. The date when hatchlings started pipping was considered the hatch date for all analyses. All hatchlings were weighed on a digital scale and morphological straight line measurements (carapace length, carapace width, plastron length, plastron width, body depth, posterior notch of plastron to vent distance, vent to tail tip distance) were taken with digital calipers to the nearest 0.01 mm within one week of hatching. Each hatchling was assigned a unique ID number which was written on their carapace with a silver sharpie to ensure individuals could be tracked throughout the experiment.

During egg collection, there was variation in clutch age at extraction, as some nests were located during or shortly after females laid eggs, while others were found an unknown number of days later. To account for this variation, for each egg, we calculated the deviation from maximum incubation length (DMIL). This variable was created using the maximum incubation length for each incubation temperature, determined from a subset of the data where it was known that eggs were extracted from the nest within 24 hours of oviposition. Then, the DMIL value was calculated for each egg that indicated how many days the observed incubation length deviated from the maximum incubation length for the given temperature at which the egg was incubated. This variable was then scaled by one standard deviation and used as a continuous covariate in some statistical models described below.

Before being assigned to their overwinter housing, hatchlings were held in individual containers with a moist paper towel. Hatchlings from 2019 were housed either in terrestrial or aquatic treatments during winter. Hatchlings from each temperature treatment were evenly distributed across the two housing treatments in a randomized fashion. Due to logistical constraints, individuals from 2020 were only housed in the aquatic treatment.

Individuals in the aquatic treatment were housed in groups of 9-10 individuals per 190 liter cattle tank, which were filled with dechlorinated water to a depth of 45 cm and included basking platforms and hiding places. These individuals were fed one pellet of Mazuri aquatic turtle diet per hatchling, three times a week. Tanks in this treatment were kept outdoors until late fall, and hatchlings were transferred from temporary housing to these tanks in batches, between August 30 and November 7 in 2019, and between October 15 and November 6 in 2020. Due to variation in hatch date and transfer date, there was variation in the time that hatchlings spent in the aquatic treatment. On November 10, 2019 and November 22, 2020, tanks were brought indoors to a temperature-controlled room that remained at approximately 22 °C, where hatchlings spent the remainder of the experiment. Tanks were brought indoors to reduce the risk of exposure to freezing temperatures, as the cattle tanks are more susceptible to rapid temperature changes than larger bodies of water. The room where tanks were housed was kept at a constant temperature due to logistical constraints. Although hatchlings were kept indoors for a large portion of the overwintering period, and winter mean daily maximum temperatures at our field site typically do not reach 22 °C, this housing treatment is not far from what natural water temperatures can be for portions of the winter in the southeast United States, and turtles may actively swim, bask, and feed when winter temperatures reach above 14 °C (Ernst, 1972; Schubauer & Parmenter, 1981). Pond sliders in South Carolina have been observed actively swimming during winter months even in air temperatures as low as 2 °C (Schubauer & Parmenter, 1981), while Suwanee cooters (*Pseudemys concinna suwanniensis*) in Florida remain active throughout the winter with water temperatures remaining around 22-23 °C (C. G. Jackson, 1970). This relatively warm temperature in the aquatic treatment was intended to ensure that hatchlings remain active and swimming throughout the winter, to provide contrast to the comparatively inactive state of the terrestrially housed hatchlings described below.

Individuals in the terrestrial treatment were kept in small containers (11 cm height x 11.5 cm diameter) filled with sand hydrated to 4% moisture by mass (Warner et al., 2024). Individuals were randomly assigned to each container, which included 7-8 individuals from multiple clutches and temperature treatments. All hatchlings from 2019 assigned to this treatment were transferred to sand containers on October 31, 2019 and kept at room temperature, then transferred to an incubator programmed to 15 °C on November 22, 2019. Incubator temperature was decreased to 10 °C on December 10, 2019 and then raised back to 15 °C on March 22, 2020. Sand tubs were transferred back to room temperature on March 28, 2020. Individuals in this treatment were not given food and were kept at a cooler temperature to facilitate lower levels of activity than the aquatic treatment, and to reflect that winter soil temperatures in nests are often cooler than water temperatures in aquatic habitats (Costanzo et al., 2008). Sand in each container was rehydrated weekly to maintain 4% hydration.

Performance trials

During the spring following hatching, righting response trials were conducted with all surviving hatchlings. Spring was selected as the best time of year to conduct trials, as all hatchlings in the wild would be active, and either already or recently emerged. Trials were conducted from March 31 to April 2, 2020, and April 13 to April 15, 2021 in a room that remained approximately 22 °C. All hatchlings were weighed and measured (straight carapace length, carapace width, plastron length, plastron width, body depth, posterior notch of plastron to vent, vent to tail tip) the day before trials. In randomized order, all hatchlings were included in three rounds of trials, one round on each of three consecutive days. For each trial, a hatchling turtle was placed in a small open-topped plastic box (15 cm wide x 20 cm long x 10 cm tall) with sand at a depth of two centimeters. Each turtle was given at least 20 minutes to acclimate and explore its container. At the beginning of each trial, each turtle was gently placed upside down on its carapace (by hand) in the middle of the container. This process was done in groups of 9 (or fewer) turtles at a time and each round was video recorded. After a group of turtles were placed upside down, researchers left the room for the remainder of the trial. All protocols in this study were approved by the Auburn University Institutional Animal Care and Use Committee (protocol number: 2019-3502).

Videos were reviewed to obtain: 1) total righting time, defined as the total amount of time it took a turtle to right itself; 2) latency to right, the time that elapsed between the turtle being placed on its carapace and its attempts to right itself; and 3) active righting time, the time the turtle spent actively attempting to right itself (equivalent to total righting time minus latency to right) (Davy et al., 2014). If a turtle was unable to right itself in 20 minutes, the time for that round was excluded from the total and active righting time datasets. Individuals that did not successfully right, but did attempt to do so were included in the latency-to-right dataset. All times were scored to the nearest second after zero, such that one second was the shortest time possible.

Statistical Analysis

To determine effects of incubation temperature on egg survival and incubation duration, we built generalized linear mixed effects models including egg survival (Binomial error and logit link function) and incubation duration (Poisson error and log link function) as response variables. DMIL was included as a continuous covariate in both models to account for variation in egg extraction date. Clutch identity was included as a random intercept in these models, and egg mass was included as an additional continuous covariate fixed effect in the incubation duration model.

To determine patterns of phenotypic variation prior to the influence of conditions during overwintering, we examined the effect of incubation temperature and egg mass on hatchling phenotypes after hatching (in the fall). We built separate univariate linear mixed effects models with each hatchling trait (carapace length, carapace width, plastron length, plastron width, body depth, posterior notch of plastron to vent distance, vent to tail tip distance) as the response variable, and incubation temperature (mean centered) and egg mass as fixed effect continuous covariates and clutch identity as a random intercept. DMIL was included as a fixed effect covariate to account for variation in egg extraction date.

A principal components analysis (PCA) was performed on all hatchling measurements taken in spring prior to trials (Table 2.1). Most of the variation in the data (>90%) was explained by PC1 and PC2, which were used in some subsequent analyses (described below). A second PCA (Table 2.2) was performed to summarize hatchling growth during overwintering. The variables analyzed in this PCA were the change in each hatchling measurement (straight carapace length, carapace width, plastron length, plastron width, mass) from fall to spring, divided by the number of days that elapsed between the two measurements; PC1 explained 92.6% of the variance in the data and was used in a subsequent analysis.

Table 2.1. Loadings from principal components analyses of spring hatchling measurements and growth.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Hatchling measurements								

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Carapace length	0.392	-0.079	0.033	-0.013	0.298	-0.328	-0.194	0.777
Carapace width	0.388	-0.105	-0.010	-0.400	0.020	-0.586	-0.239	-0.527
Plastron length	0.384	-0.170	-0.030	-0.075	0.699	0.504	0.151	-0.228
Plastron width	0.379	-0.201	0.022	-0.533	-0.561	0.379	0.181	0.199
Body depth	0.384	-0.096	0.173	0.535	-0.274	0.263	-0.604	-0.137
Notch-vent	0.186	0.816	0.521	-0.143	0.041	0.071	0.026	-0.015
Vent-tip	0.271	0.481	-0.823	0.096	-0.086	0.039	0.001	-0.001
Mass	0.386	-0.084	0.138	0.483	-0.154	-0.275	0.696	-0.084
Proportion of variance explained	0.795	0.123	0.056	0.011	0.006	0.004	0.003	0.001
Growth over winter								
Carapace length per day	0.456	-0.284	0.282	-0.173	-0.776	-	-	-
Carapace width per day	0.433	0.647	0.576	0.157	0.193	-	-	-
Plastron length per day	0.455	-0.326	0.018	-0.634	0.534	-	-	-
Plastron width per day	0.443	0.444	-0.758	-0.069	-0.162	-	-	-
Mass per day	0.449	-0.445	-0.112	0.734	0.222	-	-	-
Proportion of variance explained	0.926	0.052	0.013	0.006	0.004	-	-	-

Note: Spring measurements were taken prior to performance trials, and growth rate was calculated between fall (post-hatching) and spring measurements.

To elucidate how conditions during the embryo and overwintering treatments affected phenotypes which could be related to locomotor ability, we examined the effect of incubation temperature and the overwintering environment on hatchling phenotypes in spring. We built linear mixed effects models involving hatchling measurements (carapace length, carapace width, plastron length, plastron width, body depth), and growth PC1 as the response variables and incubation temperature, overwintering environment, and their interaction, as fixed effects. The corresponding measurement taken post-hatching (in the fall) was included as another fixed effect, as well as the number of days each hatchling spent in their overwintering housing, to account for variation in this component of the experiment. A random intercept of clutch identity was also included.

To determine factors affecting hatchling righting time, we built linear mixed effects models with response variables of total righting time, latency to right, and active righting time (all log base 10 transformed), and fixed effects that included incubation temperature, overwintering environment, the interaction of incubation temperature and overwintering environment, hatchling age at the start of trials, days spent in overwintering housing, PC1 and PC2 of spring measurements, DMIL, and year (Table 2.3S). Growth PC1 was not included as an additional fixed effect as it was strongly correlated with the PC1 of spring measurements. All successful righting attempts were included in analyses, such that 1-3 attempts were included per individual. Random effects of individual identity (to account for repeated measures) and clutch identity were included. Backward stepwise regression was used to eliminate variables in each model until only focal variables of interest (incubation temperature, overwintering environment, and their interaction) and fixed effects with a p-value of less than or equal to 0.05 remained (Tables 2.4S and 2.5S). The R packages lme4 and lmerTest were used in all described linear mixed effects models above (Bates et al., 2015; Kuznetsova et al., 2017).

The R package DHARMa (Hartig, 2024) was used to inspect residuals of each model to determine whether assumptions of a linear model were violated. Models with unusual patterns in the residuals included those with response variables of incubation duration (Figure 2.7S), carapace length (Figure 2.1S), plastron length (Figure 2.3S), and body mass (Figure 2.5S). We took steps to improve patterns of residuals in each model, including removing outliers, adding a quadratic incubation temperature term, and log transforming the response variable. With the exception of the incubation duration model, all other models showed notable improvement in the pattern of residuals following log base 10 transformation of the response variable (Figure 2.2S; Figure 2.4S; Figure 2.6S). We compared p-values obtained from the original models to those with a log transformation (Table 2.6S), and found that there were no major differences in p-value significance for any parameters, suggesting these models were robust to violations of assumptions. Therefore, we present model results from the un-transformed responses. Parameter estimates for all models can be considered reliable regardless of how closely residuals satisfy assumptions of linear models, but p-values from the incubation duration model should be interpreted with caution as all versions of this model failed to satisfy residual diagnostic tests. All data and R code used in this study are freely available on Zenodo at https://doi.org/10.5281/zenodo.18202869.

Results

Egg incubation

Incubation duration ranged from 41 to 105 days and was strongly influenced by temperature, such that lower incubation temperatures resulted in longer incubation durations (Table 2.2). Average deviations from the maximum incubation length (DMIL) at a given incubation temperature were 11.6 days (n=21), 9.5 days (n=20), 5.8 days (n=19), 5.6 days (n=18), and 4.3 days (n=14) for the 24, 26, 28, 30, and 32 °C treatments, respectively. Seventy five percent of eggs deviated 8 days or less from the maximum incubation length observed at their incubation temperature. The largest DMIL was a deviation of 39 days from the maximum incubation length of 105 days at the 24 °C temperature treatment. Egg survival was 89.9% across both years (86.7% in 2019, n=83; and 100% in 2020, n=26) and was not influenced by incubation temperature or egg mass (Table 2.2).

Hatchling survival and morphology

Turtles incubated at warmer temperatures had longer plastron lengths and shorter vent-to-tip tail measurements at hatching, but incubation temperature did not affect any other trait at hatching (Table 2.2; Figure 1S). Egg mass had a significant positive relationship with all shell dimensions and body mass (Table 2.2).

Juvenile survival to the date of their righting trials in spring was 95.8% in 2019 (n=72) and 88.5% in 2020 (n=26). All hatchlings that died were housed in the aquatic overwinter treatment (3 in 2019 and 3 in 2020). Hatchlings in the aquatic treatment gained an average of 3.1 grams in body mass and 5.9 millimeters in carapace length over winter (n=54). Hatchlings in the terrestrial treatment lost an average of 0.9 grams over winter and grew an average of 1.7 mm in carapace length (n=36). Accordingly, growth rate differed between the overwinter treatments based on analysis of the PC scores (Table 2.3). Neither incubation temperature nor the interaction between incubation temperature by overwinter environment affected growth rate (Table 2.3).

Table 2.2. The effect of incubation temperature and egg mass on egg survival, incubation duration, and hatchling phenotypes at hatching (in the fall).

Response variable	**Incubation temperature effect**	**Egg mass effect**	**DMIL effect**
Egg survival	$\beta = -0.073$ (0.118) $p = 0.540$	$\beta = -0.412$ (0.280) $p = 0.141$	-
Incubation duration (days)	$\beta = -0.078$ (0.005) $p <$ **$2.0e^{-16}$**	-	$\beta = 0.107$ (0.014) $p =$ **$5.78e^{-15}$**
Carapace length (mm)	$\beta = 0.041$ (0.030) $p = 0.183$	$\beta = 0.978$ (0.062) $p <$ **$2.0e^{-16}$**	$\beta = 0.133$ (0.131) $p = 0.319$
Carapace width (mm)	$\beta = 0.044$ (0.067) $p = 0.507$	$\beta = 0.826$ (0.116) $p =$ **$1.13e^{-6}$**	$\beta = -0.115$ (0.224) $p = 0.619$
Plastron length (mm)	$\beta = 0.111$ (0.036) $p =$ **0.003**	$\beta = 0.972$ (0.071) $p =$ **$7.32e^{-16}$**	$\beta = 0.088$ (0.146) $p = 0.554$
Plastron width (mm)	$\beta = 0.028$ (0.044) $p = 0.530$	$\beta = 0.682$ (0.086) $p =$ **$1.75e^{-9}$**	$\beta = -0.093$ (0.174) $p = 0.600$
Body depth (mm)	$\beta = 0.020$ (0.024) $p = 0.411$	$\beta = 0.345$ (0.041) $p =$ **$3.08e^{-8}$**	$\beta = -0.090$ (0.079) $p = 0.275$
Body mass (mm)	$\beta = 0.021$ (0.012) $p = 0.076$	$\beta = 0.795$ (0.026) $p <$ **$2.0e^{-16}$**	$\beta = 0.121$ (0.061) $p = 0.053$
Notch-vent (mm)	$\beta = 0.033$ (0.025) $p = 0.187$	-	$\beta = 0.165$ (0.074) $p =$ **0.042**
Vent-tip (mm)	$\beta = -0.151$ (0.035) $p =$ **$3.81e^{-5}$**	-	$\beta = 0.067$ (0.139) $p = 0.634$

Note: Clutch was included as a random effect in all models. Standard errors are noted in parentheses. The effect sizes (β) for incubation temperature can be interpreted as the change in the response variable with a one degree increase in temperature.

Both incubation temperature and overwinter environment significantly influenced post-overwintering (spring) measurements of carapace length, plastron length, and body mass (Table 2.3; Figure 2.1). Higher incubation temperatures resulted in greater carapace and plastron lengths, greater plastron widths, and greater body mass and depth in the spring. Aquatically overwintering hatchlings had greater carapace and plastron lengths and greater body mass than terrestrially overwintering hatchlings in the spring. A significant interaction between incubation temperature and overwintering environment showed that egg incubation temperature had no effect on body depth or mass for hatchlings that overwintered in the terrestrial treatment. However, incubation temperature had a positive effect on these traits for hatchlings that overwintered in water (Figure 2.1E, F). Days spent in winter housing had a significant positive relationship with all spring measurements except for mass. All previous fall measurements had a significant positive relationship with their corresponding spring measurement (Table 2.3).

Table 2.3. Effect of incubation temperature and overwintering location on hatchling phenotypes in the spring.

Phenotype	Incubation temp. effect	Terrestrial overwinter env. effect	Incubation temp. * overwinter env.	Previous measurement effect	Days in Winter Housing effect
Carapace length (mm)	β = 0.394 (0.165) p = **0.019**	β = -2.077 (0.997) p = **0.040**	β = -0.422 (0.236) p = 0.077	β = 1.370 (0.166) p = $\mathbf{2.01e^{-12}}$	β = 0.052 (0.021) p = **0.013**
Carapace width (mm)	β = 0.234 (0.180)	β = -1.401 (1.259)	β = -0.120 (0.253)	β = 0.575 (0.575)	β = 0.055 (0.025)

Phenotype	**Incubation temp. effect**	**Terrestrial overwinter env. effect**	**Incubation temp. * overwinter env.**	**Previous measurement effect**	**Days in Winter Housing effect**
	p = 0.198	p = 0.270	p = 0.636	p = **0.001**	p = **0.030**
Plastron length (mm)	β = 0.321 (0.132) p = **0.017**	β = -1.614 (0.802) p = **0.047**	β = -0.342 (0.191) p = 0.077	β = 1.168 (0.137) p = **5.9e**$^{-13}$	β = 0.034 (0.017) p = **0.044**
Plastron width (mm)	β = 0.289 (0.140) p = **0.042**	β = -0.584 (0.900) p = 0.521	β = -0.158 (0.199) p = 0.431	β = 0.895 (0.167) p = **1.7e**$^{-6}$	β = 0.049 (0.018) p = **0.009**
Body depth (mm)	β = 0.214 (0.086) p = **0.006**	β = -0.897 (0.515) p = 0.085	β = -0.300 (0.123) p = **0.017**	β = 0.991 (0.184) p = **6.22e**$^{-7}$	β = 0.033 (0.011) p = **0.002**
Body mass (mm)	β = 0.519 (0.185) p = **0.005**	β = -2.455 (1.106) p = **0.029**	β = -0.529 (0.262) p = **0.047**	β = 1.407 (0.265) p = **9.26e**$^{-7}$	β = 0.045 (0.023) p = 0.051
Growth PC1	β = 0.152 (0.100) p = 0.130	β = -1.250 (0.602) p = **0.041**	β = -0.152 (0.143) p = 0.291		β = 0.027 (0.012) p = **0.028**

Note: Random effects of clutch identity were included in all of these models. Standard errors are noted in parentheses.

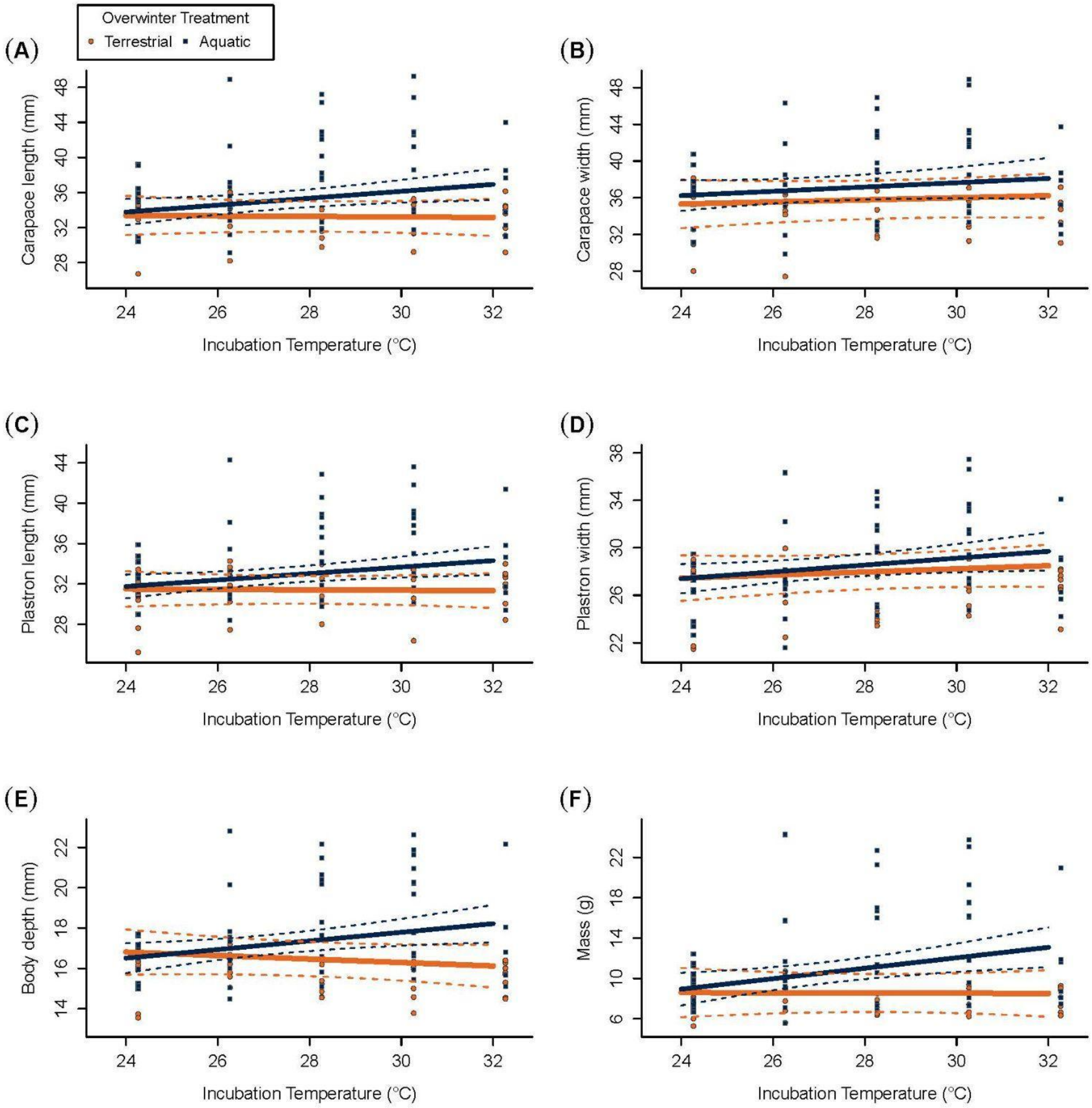

Figure 2.1. The relationships between incubation temperature and hatchling phenotypes in spring for individuals that overwinter terrestrially (orange regression line, bottom) and aquatically (blue regression line, top). Phenotypes include (A) carapace length, (B) carapace width, (C) plastron length, (D) plastron width, (E) both depth, and (F) body mass. Model predictions (solid lines) and 95% confidence limits (dashed lines) are plotted over the observed data (points) for the aquatic overwintering treatment (blue squares) and the terrestrial treatment (orange circles). Statistical results are in Table 2.3.

Righting response

Ninety-two surviving hatchlings were included in the trials, but two of these individuals were excluded from analyses due to missing morphological measurements. Sample sizes for the righting trials ranged from six to 12 individuals for each of the ten treatments (mean = 9) (Table 2.2S). Successful righting times within 20 minutes ranged from 2 seconds to nearly 20 minutes (Figure 2.2). Out of 270 attempts (3 attempts each of 90 individuals), 64 attempts from 32 individuals were unsuccessful in 20 minutes. Of these, a total of 11 individuals were not represented in the total and active righting time datasets, as all three of their attempts were unsuccessful. Data from 79 unique individuals contributed to the total righting response dataset, with an average of 2.6 successful righting times per individual. Data from 89 unique individuals contributed to the latency to right dataset, with an average of 2.9 latency times per individual. Nine attempts from six individuals were not included in the latency to right dataset, as turtles did not move or attempt to right themselves in these cases. One individual did not attempt to right itself on any attempt. Active righting time and latency to right were not correlated (r = -0.08, p=0.24).

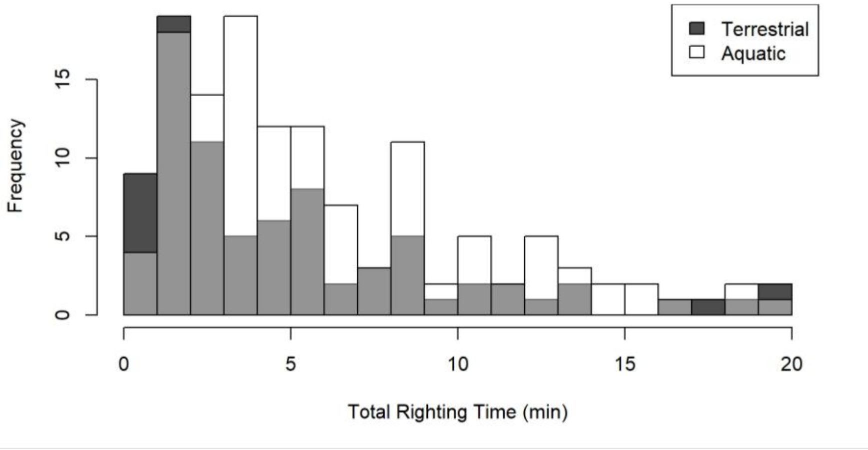

Figure 2.2. The distribution of successful righting times (within 20 minutes) for all attempts from all individuals. The terrestrial overwinter treatment is indicated in black and the aquatic treatment is in white. Gray areas on bars indicate overlap between terrestrial and aquatic treatment groups.

Incubation temperature, overwinter environment, and their interaction significantly influenced total righting time (Table 2.4). Terrestrially overwintering hatchlings had faster righting times than aquatically overwintering hatchlings, and in this treatment group, there was not a strong relationship between incubation temperature and total righting time (Figure 2.3A). Of the aquatically overwintering hatchlings, individuals from warmer incubation temperatures had longer righting times than those incubated at cooler temperatures (Figure 2.3A). Hatchlings that spent the winter terrestrially had shorter latency times than those that overwintered in water. Hatchlings with longer tails (PC2) had shorter latency times than those with shorter tails (Table 2.4). No variables included in our analyses significantly influenced active righting time.

Table 2.4. Effect of incubation temperature and overwintering location on hatchling righting performance in the spring.

Response variable	**Incubation temperature**	**Terrestrial overwinter environment effect**	**Inc. temp. * overwinter environment**	**PC2**
Total righting time (min)	β = 0.058 (0.019) p = **0.003**	β = -0.179 (0.079) p = **0.027**	β = -0.061 (0.028) p = **0.032**	
Latency to right (min)	β = 0.033 (0.024) p = 0.173	β = -0.439 (0.088) p = $\mathbf{3.49e^{-6}}$	β = -0.055 (0.030) p = 0.069	β = -0.117 (0.054) p = **0.034**

Note: The variables in the table represent those that remained in the final models after the backwards stepwise elimination procedure. Standard errors are noted in parentheses. Results from the full models can be found in Table 2.3S.

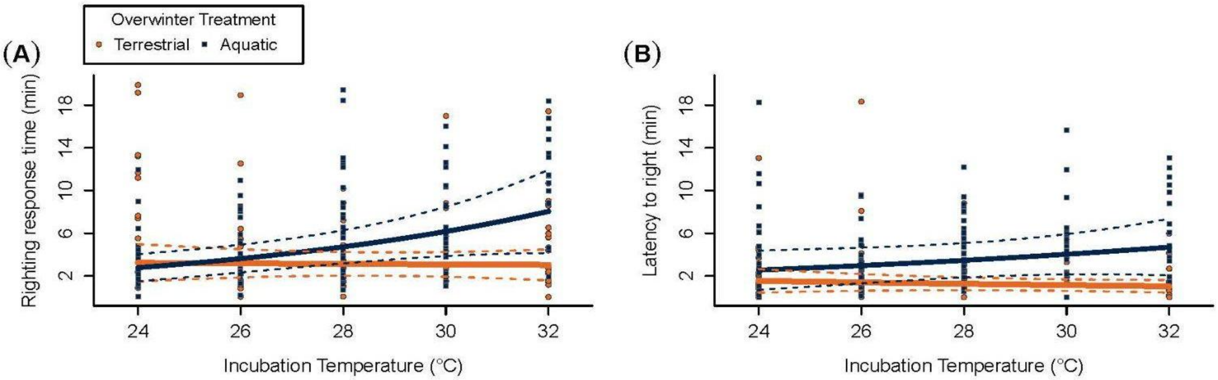

Figure 2.3. The relationships of incubation temperature with (A) total righting time and (B) latency to right for the terrestrial (orange curves, bottom) and aquatic overwintering (blue curves, top) treatments. Model predictions (solid lines) and 95% confidence limits (dashed lines) are plotted over the observed data (points) for the aquatic overwintering treatment (blue squares) and the terrestrial treatment (orange circles). Statistical results are in Table 2.4.

Discussion

A primary goal of our study was to quantify the effects of the environment during two early life stages on the morphology and performance of hatchling turtles. Our work also provides insight into the relative benefits of overwintering in two different habitats (aquatic vs terrestrial), which reflects the timing of emergence of hatchlings from their nest (fall versus spring). As demonstrated in other studies and in line with our predictions, we showed that warmer egg incubation temperatures produced larger offspring that hatched sooner than those from cooler incubation temperatures (Ewert, 1985; Georges et al., 2005; Ligon et al., 2012). The effects of incubation temperature on offspring size persisted (even strengthened) to the following spring, and individuals that overwintered on land had better righting performance than those that spent the winter in an aquatic environment, providing potential evidence of the adaptive value of remaining in the nest overwinter and emerging in spring. Importantly, the negative effect of overwintering in an aquatic environment (i.e., fall emergence) was most pronounced for those that experienced warm temperatures during egg incubation, indicating that nest temperature can affect the consequences of fall emergence from nests.

Most of the variation in hatchling morphology at the time of hatching was due to egg size, rather than incubation temperature. All of the body size variables that we measured were positively related to egg mass, but plastron length and tail length at hatching were the only variables influenced by incubation temperature. The effect of egg size is consistent with past work showing that maternal investment can have significant impacts on offspring in ways that might benefit components of fitness (Räsänen et al., 2005; Sinervo, 1990). Indeed, larger offspring often have higher survival than smaller individuals in many taxa (Bowen et al., 2015; Olsson & Madsen, 2001), including hatchling turtles (Janzen, 1993; Mitchell et al., 2013; Myers et al., 2007). Nevertheless, warm incubation temperatures still produced offspring with larger plastron lengths and shorter tails. While these effects on size might benefit individuals that emerge in the fall soon after hatching (e.g., Janzen et al., 1993), the effects on overwinter survival for those that remain inside nests during winter are unclear. Intriguingly, we show here that the influence of incubation temperature on offspring size became even more apparent the following spring, which is a time when most hatchlings emerge from nests.

Nearly all measurements of hatchling size in the following spring were influenced by incubation temperature, indicating a delayed effect of the thermal environment during embryo development. Several of these body size variables were also influenced by the overwinter environment, as well as its interaction with incubation temperature.

Hatchlings that overwintered in water were heavier and had a greater carapace and plastron length than hatchlings that overwintered terrestrially; although nest effects contribute to variation in body mass change during winter in hatchling painted turtles (Mitchell et al., 2013), and overwinter conditions can affect body condition in other reptiles (Brischoux et al., 2016), the pattern we observed likely reflects increased hydration and increased food intake/growth of turtles housed in the aquatic treatment during winter. The phenotypic effects of overwinter treatment were also influenced by incubation temperature in that hatchlings housed aquatically, but not terrestrially, during winter exhibited a greater body depth and mass if they experienced warm temperatures during egg incubation. The physiological mechanism that might generate an interaction between egg incubation temperature and overwinter location is unclear and warrants more investigation, but this pattern may be explained by how temperature during early life stages (embryonic and posthatching) affects metabolic or hydric physiology (Ben-Ezra & Burness, 2017; Booth et al., 2000; DuRant et al., 2012; Sun et al., 2015).

Terrestrially overwintering hatchlings performed better than those that overwintered aquatically. This result is not surprising in part, since most individuals of *T. scripta* have a terrestrial overwintering strategy, implying that selection must favor this behavior because it positively affects fitness. We pose several potential explanations as to why turtles that overwintered on land performed better, which could be due to metabolic or morphological effects of the overwinter habitat. First, nutritional status influences righting time in red-eared sliders, and more recent feedings result in longer latency and righting times (Delmas et al., 2007), which may partially explain why aquatic hatchlings in our study (which were fed) exhibited slower performance. Second, high temperatures generate higher metabolic demands in dormant overwintering turtles, resulting in fitness costs at emergence (Muir et al., 2013). Indeed, water temperature in our study was warmer than that in the sand substrate of the terrestrial treatment; this difference in aquatic versus nest temperature is similar in nature, particularly in northern latitudes. However, it is unclear whether these metabolic costs would translate to active hatchlings overwintering in water. The housing temperature differences alone, rather than overwintering substrate, could have driven some of the differences we observed in hatchling performance. Third, terrestrial hatchlings were smaller than aquatic hatchlings in carapace and plastron lengths, and body mass in spring when they would normally disperse from their nest. Although body size did not directly affect performance in our analyses, some aspects of the smaller size of terrestrial hatchlings could potentially improve performance. For example, previous studies have shown that smaller hatchlings are better able to right themselves (Ruhr et al., 2021). Turtle shell and body proportions can also change as body size increases (Myers et al., 2007; Ruhr et al., 2021), and shell shape may influence righting ability (Domokos & Várkonyi, 2007). Overall, our results suggest terrestrial overwintering is a safer strategy that may buffer potential individual differences in performance that became evident in the aquatic hatchlings.

Effects of incubation temperature and overwintering treatment on righting performance reflected several of the patterns observed for morphological measurements, which indicates that some aspects of hatchling morphology could have influenced the variation in hatchling performance we observed. However, while this comparison implies that larger body size may be associated with reduced performance, we found no association between these variables in our analyses. Nevertheless, incubation temperature was important to hatchling performance for aquatically overwintering individuals, but not for those that overwintered terrestrially. Of the hatchlings in the aquatic treatment, those from cooler incubation treatments performed better than those from warmer treatments. Those incubated at cooler temperatures also had a longer incubation duration, as expected based on patterns observed in other reptiles (Ewert, 1985; Georges et al., 2005). Although there was variation in lay date, for the most part, cooler-incubated individuals hatched later in the season, meaning they were younger (and perhaps smaller) at the time of the righting trials. However, we found neither an effect of age on righting time nor an effect of overall body size (PC1). Additionally, growth PC1 was highly correlated with body size PC1, suggesting that growth rate was also not related to righting time. Other factors not measured in this study may be responsible for the poorer performance of warm-incubated aquatic hatchlings when compared to their cool-incubated counterparts, such as greater usage of residual yolk and longer periods of heightened metabolism prior to winter (Muir et al., 2013; Warner et al., 2024), or longer periods of feeding. It is also possible that prolonged embryonic development of the cooler incubated eggs had a positive effect on hatchling locomotor performance due to increased opportunity for neuromuscular development, which is consistent with research on skinks (Madsen & Shine, 1999; Shine & Olsson, 2003). Lastly, the benefits of prolonged development may only become apparent in hatchlings exposed to suboptimal winter conditions, such as an aquatic habitat, which could explain the lack of an effect in hatchlings that overwintered terrestrially, and performed better overall. While other studies have demonstrated effects of incubation temperature on righting responses of hatchling turtles (Elnitsky & Claussen, 2006; Fisher et al., 2014; Micheli-Campbell et al., 2011; Riley, Freedberg, et al., 2014), we show that this effect can depend on the habitat during the overwintering stage prior to emergence, which highlights how important aspects of development continue to remain sensitive to the environment even after hatching.

Though with less statistical support, we found that latency to right mirrored the patterns described above. Hatchlings that overwintered in water had longer latency times than those that overwintered on land, and they exhibited a stronger relationship between latency time and incubation temperature. As latency represented the time a turtle took to move after being handled, latency could be associated with boldness. Indeed, rates of movement are associated with individual boldness in other animals (A. D. Wilson & Godin, 2010). Additionally, incubation temperature can affect boldness and other behaviors in reptiles (de Jong et al., 2022; Siviter et al., 2017). Acclimation to humans could also potentially explain treatment differences in latency. However, our results do not support this explanation, as aquatic hatchlings (which had longer latency times) were exposed to humans more frequently (during feedings and tank maintenance) than the terrestrial hatchlings (brief weekly sand tub rehydration). Alternatively, variation in latency could simply be associated with reaction time and coordination. The conditions terrestrial hatchlings experienced could have led to phenotypic changes that better equipped them to respond quickly after being turned over. However, as latency was not correlated with active righting time, there is no indication that individuals who quickly attempted to right could also quickly succeed in righting themselves. Additionally, hatchlings that had longer tails (PC2) had faster latency times, although it is unlikely tail length directly contributed to variation in latency to right.

An important caveat associated with our study design is that our overwintering treatments differed in a range of variables (in addition to substrate and temperature described above) that could have led to performance differences in aquatic versus terrestrial hatchlings. For example, light exposure and humidity were greater in the aquatic treatment than the terrestrial treatment. Additionally, acclimation or familiarity to the conditions during the righting response trials differed between individuals in our two overwinter treatments. While terrestrial hatchlings were already familiar with the sand substrate used during the righting trials, they also had to adjust to the room temperature prior to our performance tests, unlike those from the aquatic treatment. The timing of our trials is also important, as righting behavior of *T. scripta* is influenced by the seasonal timing of reproduction (Nichols et al., 2019), and righting performance prior to overwintering may differ from post-overwintering performance (Carter et al., 2016). We chose to measure righting behavior in the spring for both overwintering treatments to avoid confounding hatchling age with season, and also because this time of year is when most hatchlings are active and dispersing. Importantly, however, hatchlings that have already entered the water in fall are not likely to disperse over land again in spring, and may have reduced terrestrial performance after spending time in water. Nevertheless, emydid turtles retain the ability to right throughout their lives (Smith et al., 2017) and often disperse over land multiple times at older life stages (Gibbons, 1970, 1986), meaning the righting response may remain ecologically relevant and indicative of neuromuscular coordination regardless of timing.

Our study raised several questions that could form the basis for future studies that improve our understanding of overwintering behavior in relation to hatchling turtle performance. Future studies that repeatedly assess performance in the same individuals before and after winter (as in Carter et al., 2016) would provide additional insight into survival probabilities of hatchlings that emerge at different times of year. Incorporating differing overwintering habitats in these experiments would be an informative next step. These studies would also benefit by mimicking the spatial and temporal variation of temperature in natural overwintering sites (e.g., Warner et al., 2024). Importantly, these studies must consider that pond sliders in lower latitudes often have bouts of swimming activity during winter interspersed with long periods of cold and inactivity (Costanzo et al., 2008; Schubauer & Parmenter, 1981). Thus, studies that implement natural thermal variation in the aquatic environment will provide a more ecologically-relevant understanding of the impacts of aquatic overwinter sites. Another factor that should be considered is that some hatchlings may emerge from the nest in the fall, but overwinter outside the nest cavity on land (Gibbons, 2013). Determining the factors that influence hatchling decisions about when to emerge and where to spend winter will shed more light on the complexity of this unique stage in juvenile turtles.

Conclusions

This study provides insight into the importance of thermal and hydric characteristics during multiple early life stages of hatchling pond sliders. Our results indicate that terrestrial overwintering, most often associated with spring emergence, produces hatchling phenotypes associated with greater neuromuscular coordination, highlighting the adaptive value of spring emergence. Additionally, the effects of incubation temperature for hatchlings that overwinter in water emphasizes the importance of the interaction between these two early life stages. Warm nest temperatures combined with warm conditions and high metabolic demands during the overwintering period may result in hatchlings with reduced coordination, potentially leading to lower survival. Altogether, our findings highlight the sensitivity of turtles to environmental alterations, as early life conditions are crucial in determining whether a hatchling may survive post emergence. We have shown that conditions at the nest site can have important consequences not only for embryonic development, but also for hatchling turtles over winter and after emergence, which highlights the need for turtle conservation programs that consider terrestrial environmental quality in addition to aquatic habitat. Gaining a greater understanding of how early life conditions may impact turtle populations is especially important, as turtles have slow population recovery due to delayed sexual maturity and low juvenile survivorship (Congdon et al., 1993). Furthermore, as anthropogenic changes to natural habitats and to climate increase, it is crucial to understand the damaging effects these alterations could pose both to early development and to turtle populations as a whole.

Acknowledgments

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Ethics Approval Statement

This work was approved by the Auburn University Institutional Animal Care and Use Committee (protocol number: 2019-3502).

Chapter 3: Observed and estimated pond slider (*Trachemys scripta*) sex ratios across an urbanization gradient

Humans impact wildlife in a variety of ways that have negative influences on populations and entire ecosystems. Anthropogenic changes such as habitat fragmentation and destruction, urbanization, human disturbance, climate change, and harvesting can impact multiple aspects of populations (C. R. Brown & Bomberger Brown, 2013b; Cordonnier et al., 2019; Habib et al., 2007; Ruell et al., 2012; Stoner et al., 2006; Vetter et al., 2020). The way that urbanization specifically impacts wildlife populations has been extensively studied in some taxa. Changes associated with urbanization such as an increase in impervious surfaces in an area can drastically alter the landscape and can cause changes to foraging behavior (Vega Rabelo et al., 2025), species assemblages and abundances (Geslin et al., 2016), while increases in housing density are often associated with increased human-wildlife interactions (Kretser et al., 2008).

Increased road density, typically associated with increased urbanization, can have profound impacts on species that must move across the landscape (Bennett, 2017). In addition to causing high rates of mortality in urban areas, road mortality can in some cases lead to changes in mean trait values through rapid evolution (C. R. Brown & Bomberger Brown, 2013b), or can differentially impact the sexes, age classes, or other categories of individuals in a population. For instance, male blue-black grassquits (*Volatinia jacarina*) experience more road mortality than females, likely due to increased collisions during territorial and mate-searching behaviors (Vianna et al., 2023). Sex-based differences in larval dispersal in the European common glow-worm (*Lampyris noctiluca*) result in female-biased road mortality (Lehtonen et al., 2021). Altered non-primary sex ratios can influence aspects of a population ranging from mate seeking behaviors to parental care (Ancona et al., 2017). Populations that are male-biased may experience an increase in aggressive mating behaviors that are harmful to females (Le Galliard et al., 2008). While studies in many taxa show that a female-biased population can be beneficial for reproductive output, populations that are extremely female-biased may experience issues such as the inability of females to locate mates when they are fertile (Courchamp et al., 1999; Freeman et al., 2014).

The impact of urbanization on freshwater turtle populations is a topic that has received considerable attention in recent decades. Both male and female freshwater turtles must travel over land for a variety of reasons (Gibbons, 1970, 1986). Turtles of either sex often disperse across land to suitable habitats, to seek mates, or to find terrestrial overwintering sites (Gibbons, 1986). However, for all female freshwater turtles, traveling on land to build a nest is a central aspect of their life history that may make them disproportionately vulnerable to the dangers of roads (Steen et al., 2006; Steen & Gibbs, 2004), and there is a growing body of evidence related to female-biased road mortality in urban areas. However, there are mixed results in the literature and knowledge gaps that still exist. Some studies have shown increased urbanization is strongly associated with male-biased populations (Steen & Gibbs, 2004), while some have shown no relationship (Vanek & Glowacki, 2019), and others have shown an opposite trend toward female-bias (Bowne et al., 2018; Lambert & Steen, 2019). Additionally, findings among various studies on the topic are not easily comparable, as differing urbanization metrics, experimental designs, and statistical approaches are used.

In addition to road mortality, there are other potential reasons we may expect skewed sex ratios as a result of urbanization. Female turtles may be disproportionately affected by urbanization through an increase in the abundance of mesocarnivores in urban areas. As females must spend long periods immobile on land in order to nest, they are more susceptible than males to predators whose population densities are often positively affected by urbanization, such as raccoons (Feinberg & Burke, 2003b; Refsnider et al., 2015). Conversely, the predation release hypothesis suggests that road densities may benefit some prey species whose predators may be excluded from urban areas (Downing et al., 2015; Johnson & Collinge, 2004). Additionally, as turtles have temperature-dependent sex determination (Type Ia and Type II, (Ewert & Nelson, 1991), the urban heat island hypothesis suggests that increased temperatures in urban areas may increase the frequency of female-producing nest temperatures , and thus create a female-biased sex ratio (Janzen, 1994a; Roberts et al., 2023; Schwanz et al., 2010). Other factors such as the proximity of suitable nest sites to aquatic habitats may come into play when considering how greatly female turtles may be impacted by road density.

Despite the abundance of studies on the topic (see Table 3.1), there are still knowledge gaps that need to be addressed. The majority of studies on urbanization and freshwater turtle population structure have taken place over short time frames (DeCatanzaro & Chow-Fraser, 2010; Dorland et al., 2014; Steen & Gibbs, 2004), some with limited trapping effort, mostly within the northern United States and Canada (Auge et al., 2024; DeCatanzaro & Chow-Fraser, 2010; Dorland et al., 2014; Dupuis-Désormeaux et al., 2019; Marchand & Litvaitis, 2004; Patrick & Gibbs, 2010; Reid & Peery, 2014; Steen & Gibbs, 2004; Vanek & Glowacki, 2019). Additionally, many studies sample over a large geographical area (Bowne et al., 2018; Roberts et al., 2023), making findings more generalized, and increasing the likelihood of confounding factors related to variation in geographic location. Moreover, some studies have taken place in areas that are potentially too urban to detect any effect of differences in road density (Vanek & Glowacki, 2019). Additionally, most of these studies have focused on just two species (*Chrysemys picta* or *Chelydra serpentina*; Table 3.1), which limits our general understanding of urbanization across different species.

In the majority of previous studies, sampled sex ratios from trapped individuals are used to assess the relationship of sex ratio with various urbanization metrics, overlooking the potential for biased results when using observed counts. Sampled sex ratios represent a subset of the population that may not accurately reflect the true proportion of males and females in the population. Although some urban turtle population studies have used capture-mark-recapture models (Auge et al., 2024; Stemle & Searcy, 2024), none to our knowledge have directly assessed estimates of male and female population size in relation to variables associated with urbanization. Capturing turtles in baited hoop traps is thought to result in a male-biased sample (Ream & Ream, 1966), but whether a trapped subset of the population is truly male-biased, or the degree to which it is male-biased may vary across sites and habitat types. Previous studies have suggested that males are more frequently captured in hoop traps than females, but that this sampled male bias should be constant across different urbanization levels (Steen & Gibbs, 2004; Vanek & Glowacki, 2019), while others show that capture method has no significant effect on broadscale sex ratio patterns (Gibbs and Steen 2005). However, a more accurate approach to assess sex ratios would involve using capture-mark-recapture models (White & Burnham, 1999) to estimate sex-specific population sizes. Proper sampling and model design along with the consideration of assumptions associated with capture-mark-recapture models can aid future work in avoiding problems associated with sampling biases.

Reporting sampled sex ratios rather than population size estimates is often simpler due to the many difficulties that come with estimating demographic parameters in natural populations using mark-recapture data, including emigration and immigration, mortality, capture biases, etc. In addition, sex ratio estimates may be influenced by an unequal probability of recapture between the sexes (Ancona et al., 2017). However, moving toward an emphasis of more rigorous sampling that allows for more precise calculations of sex ratios will improve the replicability of studies in this field and increase the strength of future findings.

Our aim is to quantify relationships between population sex ratios and level of urbanization in the pond slider (*Trachemys scripta*) a species of turtle that has been relatively understudied in this field. Moreover, we compare adult sex ratios based on our sampling effort to those estimated via mark-recapture to determine how these different metrics relate to urbanization. We predicted that the level of urbanization will influence adult sex ratios and specifically that areas with higher levels of urbanization will be associated with more male-biased populations. We also predicted that our sampled sex ratios would significantly differ from those derived from capture-mark-recapture models, which can impact how we interpret the influence of urbanization on population sex ratios.

Table 3.1. Previous peer-reviewed studies involving North American freshwater turtle sex ratios in relation to varying levels of urbanization.

Species	Area	Years in study	# Sites	Effort	Effort per site	# Turtles	Study
Pseudemys floridana, Trachemys scripta, Sternotherus odoratus, Apalone spinifera	FL, USA	4	–	5664 hours	–	4691	Aresco 2004
Chrysemys picta	NH, USA	2	37	~1480 trap nights	~ 40 trap nights	1342	Marchand & Litvaitis 2004
Chrysemys picta, Chelydra serpentina	NY, USA	1	35	420 trap nights	12 trap nights	230	Steen & Gibbs 2004
Chrysemys picta	ON, Can.	1	2	–	4 trap nights	137	DeCatanzaro & Chow-Fraser 2010
Chrysemys picta, Chelydra serpetina	NY, USA	3	15	1772 trap nights	118 trap nights	327	Patrick & Gibbs 2010
Chrysemys picta	ON, Can.	1	20	120 sampling days	6 sampling days	554	Dorland et al. 2014
Emydoidea blandingii, Chrysemys picta, Chelydra serpentina	WI, USA	3	18	–	–	1244	Reed & Peery 2014

Species	Area	Years in study	# Sites	Effort	Effort per site	# Turtles	Study
Chrysemys picta	11 U.S. states	2	26	416 trap nights	16 trap nights	–	Bowne et al. 2018
Chrysemys picta, Chelydra serpentina	ON, Can.	2	–	–	–	320	Dupuis-Désormeaux et al. 2019
Chrysemys picta	IL, USA	8	36	11,924 trap nights	331 trap nights	2685	Vanek & Glowacki 2019
Clemmys guttata	MN to FL, USA (12 states)	3	58 plots	–	20-60 trap nights	1169	Roberts et al. 2023
Emydoidea blandingii	ON, Can.	7	2	–	–	128	Auge et al. 2024
11 species	FL, USA	3	17	~680 trap nights	~40 trap nights	215	Stemle & Searcy 2024

Methods

Study Sites

We collected data at a set of five sites along a gradient of urbanization that ranged from natural to suburban in Lee, Chambers, and Macon counties, Alabama, USA (Table 3.2). Each site contained a single pond between 4,500 to 75,000 m^2 in area. Pond 1 is a naturally formed pond surrounded on one side by forest and the other by a pebbly beach. Pond 2 is located on private property and is mostly surrounded by wooded areas. Pond 3, a fisheries pond, is surrounded by grassy areas and other fisheries ponds. Pond 4, near Auburn University's campus, is surrounded by a walking path, and is close to roads, parking lots, and campus buildings. Pond 5, located at a city park, contains a dock, walking paths, benches, and exercise equipment, and is near multiple residential areas. With the exception of Pond 1, all other ponds are manmade.

Turtle Trapping

We collected data on captured turtles from 2019 to 2025. The effort and the frequency of visits to each site varied (Table 3.2). Site 4 was more extensively sampled in comparison to the other sites due to its involvement in another long-term turtle population study. Traps were typically set each year between mid May and early August, although trapping at other times of year occasionally took place (especially at Pond 4). For each trapping session, traps were typically set for a period of two to three trap nights, and were always checked at least once every 24 hours. Hoop traps were set so that they were only partially submerged, and floats were included in each trap. In order to reduce capture biases, a variety of trapping methods were used. A combination of several large (76-92 cm diameter, some with 30 or 50 meter lead nets) and small (31 cm diameter) hoop traps were typically used for each trapping session, along with the occasional use of basking traps, hand captures, and seining. Mesh size in our smallest traps was small enough to prevent the escape of turtles as small as hatchlings, while our largest traps had funnel sizes large enough to allow for the capture of adult common snapping turtles (*Chelydra serpentina*). Traps were typically baited with canned sardines, but occasionally were set without bait or with bait other than sardines. Morphological measurements (straight carapace length, carapace width, plastron length, plastron width, body depth, posterior notch of plastron to vent, vent to tail tip) were taken using digital calipers, and turtles were weighed using Pelosa spring scales. Individuals were given a unique mark by notching marginal scutes (F. R. Cagle, 1939), and then released. Species, sex, and age (juvenile vs. adult) were also recorded for each individual.

Table 3.2. Information associated with each site at which turtles were trapped.

Study area	**Pond Size (m^2)**	**Years of trapping data**	**Total trap nights**	**# Adult turtles**	**Percent individuals recaptured**	**Percent recapture events**
Pond 1	7,342	2019, 2021, 2022, 2024, 2025	115	37	27.0	30.2
Pond 2	74,612	2023-2024	50	27	3.7	3.6
Pond 3	11,558	2021, 2022, 2024	58	25	20.0	19.4
Pond 4	6,858	2019-2025	948	90	68.9	73.2
Pond 5	4,561	2019, 2021, 2022, 2024, 2025	41	74	18.9	18.7

Number of adult turtles is the number of adults in the dataset that were classified as adults at first capture and were of known sex and ID. “Percent recapture events” refers to how many captures out of the total number of captures of adults were from individuals captured previously. “Percent individuals recaptured” refers to how many individuals in the dataset of adults were ever recaptured.

Statistical Analysis

Several species of turtles were captured during our study (*Apalone spinifera, Chelydra serpentina, Chrysemys picta, Deirochelys reticularia, Kinosternon subrubrum, Pseudemys concinna, Sternotherus odoratus, Trachemys scripta*), but we chose to analyze data of pond sliders (*Trachemys scripta*) only, as this species was the most abundant across all sites, while other species captured had lower sample sizes and were not present at all sites. In all analyses, we used only observations of individuals that were adult or subadult and of known sex at first capture, in order to simplify mark recapture estimates. Individuals in our dataset were considered adults for the purpose of our analyses when they could be sexed using secondary sexual characteristics, and their straight carapace length was greater than 100 mm, the size at which males are typically sexually mature (F. R. Cagle, 1948; Lewis et al., 2018). Although females typically become sexually mature at larger sizes (F. R. Cagle, 1944; Lewis et al., 2018), we were able to reliably distinguish males from females at this size threshold, and thus we kept all these individuals in the dataset. We eliminated 10 individuals (out of 263 individuals) in our dataset whose unique ID could not be confirmed, or who could not be uniquely marked due to logistical constraints.

To quantify the level of urbanization at each study area, we downloaded data from the United States Environmental Protection Agency StreamCat Dataset that contains standardized measures of natural and anthropogenic features of streams, their catchments, and watersheds. We used the WATERS GeoViewer (https: //www.epa.gov/waterdata/waters-geoviewer (accessed on 20 September 2022) to select the closest stream segment to the ponds at our study areas and downloaded the StreamCat data associated with the catchment area draining into that segment. Five variables were extracted from the watershed dataset: mean imperviousness of anthropogenic surfaces within catchment, percentage of local catchment area classified as developed, average density of roads per square kilometer, mean of all housing units per square kilometer, and mean of 2010 census population per square kilometer. For ponds that were equidistant to two segments, we took the average value for each variable across both segments. We performed a principal components analysis (PCA) on these five variables across each of our study areas.

We obtained sex-specific superpopulation size estimates for each site using RMark (Laake, 2013) to analyze mark recapture data using the POPAN formulation (Schwarz & Arnason, 1996) of the Jolly-Seber model (Jolly, 1965; Seber, 1965). To create encounter histories, we pooled all captures by year. Previous studies that have sampled on a more frequent basis have pooled sampling occasions by year (Kemp, 2022), and this methodology is thought to reduce bias by maximizing the sample size for each sampling occasion (O'Brien et al., 2005) and to be suitable for turtles due to their long lifespans and low recruitment rates (Kemp, 2022; Reinke et al., 2019). We built a set of models with parameters (survivorship, capture probability, probability of entry, and superpopulation size) that were either constant or that varied by sex (Tables 6). Although not all assumptions of open population mark recapture models are met in our analyses, violations associated with pooling sampling occasions are typically robust to these violations (O'Brien et al., 2005), and we provide these estimates primarily to show how sampled sex ratios may differ from population size estimates.

A chi-square goodness of fit test was used to determine if sex ratios (both sampled numbers and population size estimates) differed from parity. A set of generalized linear models (binomial error and logit link function) that included the proportion of males and females as the dependent variable were used to determine relationships between sex ratios and the level of urbanization. The proportion at each site was considered an individual data point. In order to assess how statistical results may differ between studies using sampled numbers of individuals versus those using population size estimates, we ran one set of models using sampled (raw) numbers of males and females, and another using male and female population size estimates, as the dependent variable. Additionally, in order to address disparities across studies that analyze urban metrics separately versus those that conduct PCAs on a number of urbanization variables (see (Lambert & Steen, 2019), we ran one set of models using

PC1 (urbanization) and PC2 (high imperviousness and low housing) from the urbanization PCA as independent variables, and another using metrics of urbanization obtained from EPA's StreamCat dataset. For all models described above, to avoid over parameterization, we chose to run multiple models with one fixed effect each, and include AICc values to address model fit, rather than running one model that included multiple fixed effects.

Results

Principal Components Analysis

The first principal component (PC1) explained 92.6 percent of the variation in the data, while PC2 explained 4.3 percent. High positive values of PC1 represent high levels of all urbanization variables considered. High positive values of PC2 represent high levels of imperviousness and low numbers of housing units (Table 3.3, Table 3.4). PC3, PC4 and PC5 were not used in any analyses, as they explained very little of the variation in the data.

Table 3.3. Loadings from a PCA on variables associated with urbanization at the five study sites. We multiplied PC scores by negative one to facilitate ease of interpretation so that larger positive PC scores indicate higher levels of urbanization.

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Imperviousness	0.442	0.621	0.258	−0.092	0.586
% Developed	0.464	0.019	0.061	−0.721	−0.511
Road Density	0.437	0.031	−0.881	0.171	0.052
Housing Units	0.431	−0.778	0.208	−0.013	0.406
Population	0.460	0.084	0.332	0.665	−0.478
Proportion of variance	**0.926**	**0.043**	**0.030**	**3.900e-4**	**0.000**

Table 3.4. Urbanization variables and principal component scores associated with each site.

Site	Imperv.	% Devel.	Road Density	Housing Units	Popn.	PC1	PC2	PC3
Pond 1	0.085	2.068	0.913	3.253	6.200	−1.973	−0.159	0.576
Pond 2	0.430	7.270	3.132	3.119	5.978	−1.429	−0.099	−0.333
Pond 3	2.395	15.789	3.489	9.212	24.583	−1.092	0.003	−0.378
Pond 4	18.243	56.443	5.005	71.675	428.622	1.438	0.760	0.101
Pond 5	17.433	81.742	6.534	245.472	601.196	3.055	−0.506	0.034

Sampled and Estimated Sex Ratios

According to sampled numbers of turtles, our most male-biased population was at our second most urban site, Pond 4, while our most female-biased population was at Pond 2 (Table 3.5, Figure 3.1). At Pond 4, 67.8%of sampled adults were male, while at our most urban site (Pond 5), only 47.3% of sampled adults were male (Table 3.5, Figure 3.1). According to population size estimates, our most male-biased population was at our least urban site (Pond 1), and our most female-biased population was at our most urban site (Pond 5) (Table 3.5, Figure 3.1). In terms of sampled numbers of individuals, the only site which had a sex ratio that significantly differed from parity was Pond 4 (Table 3.5). When considering population size estimates, sex ratios at all sites significantly differed from parity (Table 3.5).

Table 3.5. Sampled (raw) numbers of adult males (M) and females (F), and population size estimates of adult males and females (rounded to the nearest whole number) at five focal sites. Population size estimates were obtained using a model in which population size and capture probability were allowed to vary by sex, and other

parameters (apparent survival and probability of entry) were kept constant (see model 3 in Table 3.6). Standard errors for population size estimates are in parentheses. Chi-square values from a goodness of fit test to determine whether sex ratios at each site differed from parity are also included (df = 1).

	Sampled numbers				**Population size estimates**			
Site	**M**	**F**	**% M**	**χ^2**	**M**	**F**	**% M**	**χ^2**
Pond 1	23	14	62.1	χ^2 = 2.189 p = 0.139	119 (63.972)	25 (7.110)	82.6	χ^2 = 61.361 **p = 4.751e-15**
Pond 2	12	15	44.4	χ^2 = 0.333 p = 0.564	NA*	NA*	NA*	NA*
Pond 3	16	9	64.0	χ^2 = 1.960 p = 0.162	31 (12.889)	16 (8.488)	66.0	χ^2 = 4.787 **p = 0.029**
Pond 4	61	29	67.8	χ^2 = 11.378 **p = 7.433e-4**	75 (4.605)	35 (2.891)	68.2	χ^2 = 14.545 **p = 1.368e-4**
Pond 5	35	39	47.3	χ^2 = 0.216 p = 0.642	114 (38.900)	150 (54.415)	43.2	χ^2 = 4.909 **p = 0.027**

*Only sampled numbers are reported for Pond 2, as population size estimates could not be obtained due to a lack of recaptures at this site.

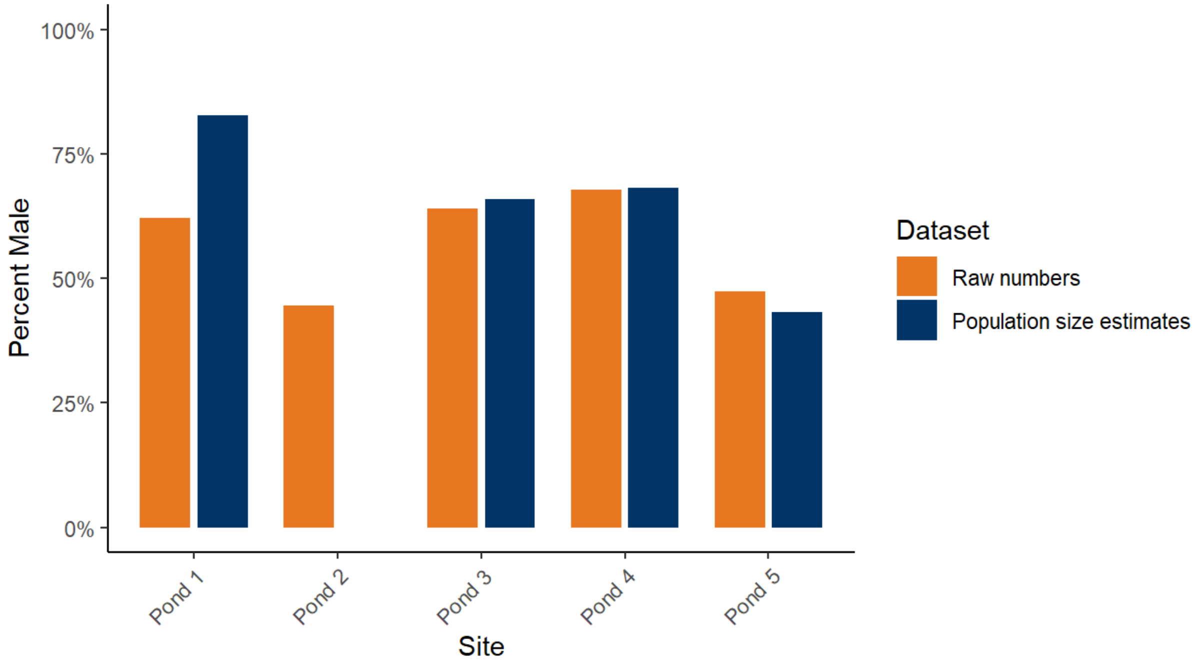

Figure 3.1. Percent male at each site, increasing in urbanization (PC1) from left to right, both from raw data and population size estimates. Bar for raw numbers (orange) are shown to the left of bars for population size estimates (blue).

POPAN Models

We were able to obtain population size estimates for males and females at each site (Table 3.5) other than for Pond 2, at which we did not have any recaptures between the two study years for which we have data at this site. For Pond 2, we present the model selection table (Table 3.6), but note that population sizes returned from these models match the raw numbers in our data. There was variation in which of the POPAN models was the most parsimonious for each site (Tables 6). However, for all sites, models 1, 2, and 3 were within 4 AICc of each other, meaning there is comparable support for all of those models (Burnham & Anderson, 2002). Therefore, we chose to use population size estimates obtained from model 3 (constant survivorship, capture probability varying by sex, constant probability of entry, and population size varying by sex) for use in our generalized linear models, as we aimed to allow estimated sex ratios to differ from our sampled sex ratios.

Table 3.6. Jolly-Seber POPAN models from each site, involving parameters that are either constant or vary by sex. Models built using RMark in R (cite). (φ = apparent survival, p = capture probability, pent = probability of entry, N = superpopulation size)

Dataset	**Models**	**Parameters**	**AICc**	**Delta AICc**	**Weights**
Pond 1	Model 3: φ(1)p(sex)pent(1)N(sex)	6	154.531	0.000	0.501
	Model 2: φ(sex)p(1)pent(1)N(sex)	6	156.517	1.986	0.186
	Model 1: φ(1)p(1)pent(1)N(sex)	5	156.523	1.991	0.185
	Model 4: φ(sex)p(sex)pent(1)N(sex)	7	157.249	2.718	0.129
Pond 2	Model 1: φ(1)p(1)pent(1)N(sex)	5	18.871	0.000	0.711

Dataset	Models	Parameters	AICc	Delta AICc	Weights
	Model 2: φ(sex)p(1)pent(1)N(sex)	6	22.213	3.343	0.134
	Model 3: φ(1)p(sex)pent(1)N(sex)	6	22.213	3.343	0.134
	Model 4: φ(sex)p(sex)pent(1)N(sex)	7	25.908	7.038	0.021
Pond 3	Model 1: φ(1)p(1)pent(1)N(sex)	5	81.666	0.000	0.520
	Model 2: φ(sex)p(1)pent(1)N(sex)	6	82.928	1.262	0.277
	Model 3: φ(1)p(sex)pent(1)N(sex)	6	84.808	3.142	0.108
	Model 4: φ(sex)p(sex)pent(1)N(sex)	7	85.070	3.404	0.095
Pond 4	Model 2: φ(sex)p(1)pent(1)N(sex)	6	436.802	0.000	0.424
	Model 1: φ(1)p(1)pent(1)N(sex)	5	437.446	0.644	0.307
	Model 4: φ(sex)p(sex)pent(1)N(sex)	7	438.721	1.920	0.162
	Model 3: φ(1)p(sex)pent(1)N(sex)	6	439.569	2.767	0.106
Pond 5	Model 1: φ(1)p(1)pent(1)N(sex)	5	230.704	0.000	0.567
	Model 3: φ(1)p(sex)pent(1)N(sex)	6	232.865	2.161	0.192
	Model 2: φ(sex)p(1)pent(1)N(sex)	6	233.009	2.305	0.179
	Model 4: φ(sex)p(sex)pent(1)N(sex)	7	235.157	4.453	0.061

Sampled Sex Ratio Models

In models using sampled proportions of males as the dependent variable, the only fixed effect that significantly influenced the proportion of males was PC2 (high imperviousness and low housing) (Table 3.7).

Table 3.7. Results of generalized linear models, using sampled proportions of males and females (raw data) as the dependent variable, and principal component scores and urbanization variables associated with each site as independent variables.

Fixed effect	Effect Size	Standard Error	p-value	AICc	Delta AICc
PC2	0.647	0.249	**0.010**	33.759	0.000
Housing units	-0.002	0.001	0.085	37.700	3.940
Road density	-0.064	0.069	0.350	39.788	6.029
PC1	-0.050	0.067	0.454	40.106	6.346
% Developed	-0.003	0.004	0.501	40.213	6.454
Population	-2.819e-4	0.001	0.587	40.372	6.613
Imperviousness	0.005	0.016	0.737	40.556	6.796

Estimated Sex Ratio Models

In models using proportions of males obtained from population size estimates as the dependent variable, we found a significant effect of all principal component scores and variables associated with urbanization (imperviousness, percent developed, road density, housing units, population). There was a negative relationship of the proportion of males to PC1 (urbanization) and all urbanization variables analyzed separately, while there was a positive relationship of the proportion of males to PC2 (high imperviousness and low housing) (Table 3.8).

Table 3.8. Results of generalized linear models, using proportions of males and females obtained from POPAN model 3 from each site (Table 3.6) as the dependent variable, and principal component scores and urbanization variables associated with each site as independent variables.

Fixed effect	Effect Size	Standard Error	p-value	AICc	Delta AICc
Road density	-0.300	0.042	**1.220e-11**	47.912	0.000

Fixed effect	**Effect Size**	**Standard Error**	**p-value**	**AICc**	**Delta AICc**
Housing units	-0.006	0.001	**1.270e-12**	51.820	3.908
PC1	-0.288	0.042	**1.220e-11**	54.870	6.958
% Developed	-0.018	0.003	**2.040e-11**	56.084	8.172
Population	-0.002	3.393e-4	**1.150e-10**	60.109	12.197
Imperviousness	-0.060	0.011	**3.190e-8**	72.386	24.474
PC2	0.854	0.199	**1.850e-5**	85.143	37.231

Discussion

In summary, we found mixed results in our analysis of the relationship of pond slider sex ratios to urbanization, but general trends did not follow our predictions. We found that when combining all our data, populations within our study area are male-biased overall. However, we found the opposite of what we expected at both ends of the urbanization gradient that we sampled. When considering population size estimates, we found that our most urban site was female-biased, while our least urban site was strongly male-biased. We found significant relationships of the proportion of males to a number of variables, including both principal components, and direct measures of urbanization. However, statistical relationships were often in the opposite direction than we predicted.

The significant positive effect of PC2 (high imperviousness and low housing) on proportions of males highlights the differences in our two most urban sites, and may explain why drastic differences in sex ratios were observed in these two seemingly similar sites that are also close geographically. Pond 4 has a very low housing density and a slightly higher impervious surfaces, while Pond 5 has a much higher density of houses than Pond 4. When housing density was analyzed separately in our data, there was a strong negative relationship with the proportion of males (Table 3.8). A higher density of houses is associated with a greater area of yards with lawns, or in other words, more semi-natural surroundings that contain suitable nesting habitat. This is confirmed both by satellite imagery as well as by personal observation. Open grassy areas are used for nesting and some emydid pond turtles frequently nest in lawns (Lambert & Steen, 2019; Marchand & Litvaitis, 2004; Rose, 2011). Road density is likely not the only barrier to travel that poses dangers to nesting females, as other structures associated with impervious surfaces such as sidewalks, curbs, parking lots, and buildings may alter and lengthen the natural path a turtle takes when making overland movements. Females traveling from Pond 4 may be more likely to encounter these hazards when traveling to a suitable nest site, as opposed to females at Pond 5 that have a plentiful availability of open grassy areas for nesting. We found little evidence of nesting directly surrounding Pond 5 (Folkerts Caldwell et al., 2023), but lawns in nearby neighborhoods contain appropriate nesting habitat. Female-biased road mortality at Pond 5 in particular may be reduced due to the specific type of land cover surrounding the pond. This difference between Pond 4 and Pond 5 that is not reflected in just one general measure of urbanization highlights the importance of considering multiple variables. It is important to make a distinction between land cover types (Lambert & Steen, 2019) as areas with grass and tree cover and those with more impervious surfaces may be drastically different even if both are considered to be “developed”.

Our finding that our least urban pond (Pond 1) was the most male-biased according to population size estimates is more difficult to explain. This result could be explained by cooler nest temperatures that are often associated with less urban areas, i.e. the “heat island hypothesis” (Janzen, 1994b; Roberts et al., 2023; Schwanz et al., 2010) producing male-biased sex-ratios at hatching. However, data from a previous study show that nest temperatures at Pond 1 are warmer on average than at our other sites, possibly due to a pebbly substrate versus the soil substrate at other sites (Folkerts Caldwell et al., 2023), meaning nests there are not likely to experience lower, majority male-producing temperatures. It is possible that the presence of a nearby stream, which may have eased movements to and from this pond, affected emigration and immigration at this site, as pond sliders are known to move between lentic and lotic habitats (Bodie & Semlitsch, 2000). Females do not always return to the same pond following nesting (Rose & Manning, 1996), and it is possible that post-nesting, some females from this site may return to the stream habitat instead of the pond, or may disperse to other areas via the stream. Conversely, the ease of dispersal by stream could also facilitate immigration of more males to this site, as they are the more vagile sex (Parker, 1984). It should also be noted that our sampling at this site (as well as at others) took place primarily during nesting season, which is a time when generally more females and less males would be making overland movements, possibly causing our sampled sex ratios to be overly male-biased.

Several of our model results support the opposite of our predictions and show that higher levels of urbanization are associated with increasing proportions of females in the population. When we used population size estimates in our models, we found that PC1 (urbanization) had a negative relationship to the proportion of males, as did all variables associated with urbanization that we analyzed separately (imperviousness, percent developed, road density, housing units, population). However, we believe that the unexpected female-biased sex ratio at Pond 5 described above, is what drove these significant negative relationships. The significant positive effect of PC2 (high imperviousness and low housing) on male proportion, even in our models using raw numbers, suggests that higher housing density in this area could be responsible for this sex ratio reversal. However, there may be other explanations for the female-biased population at this site, including variables not measured in our study such as soil temperatures in surrounding neighborhoods.

However, some aspects of our results support prevailing hypotheses on female-biased road mortality. Although differences in sex ratios across sites were not consistent with increasing levels of urbanization across sites, our result of a strong male bias at one of our most urban sites (Pond 4) is consistent with the hypothesis of female-biased road mortality. Additionally, when all of our data are considered together, a male bias is shown. Previous work has suggested relatively high road densities may drive broad patterns in sex ratios, specifically that an increased fraction of males was associated with regions with road densities over 0.75 km/km^2 (Gibbs & Steen, 2005). Our overall findings support this, as even our least urban site had a road density greater than 0.75 km/km^2. However, it should also be noted that the most urban end of the gradient we sampled in is more suburban than urban. Thus, this narrow urbanization gradient could have influenced our ability to detect some trends that may occur in areas with a broader range of urbanization variables.

Besides the direct results of our statistical analyses, an important takeaway from this study is how sex ratios obtained from mark recapture models can differ greatly from sampled sex ratios. This finding was consistent with our predictions. Although no sex ratios were reversed between sampled and estimated ratios, the degree to which populations were male- or female-biased changed substantially in some cases. For instance, when using population size estimates, sex ratios at all sites differed from parity, while only one site had a sampled sex ratio that differed from parity. Our most urban site (Pond 5) was estimated to be even more female-biased than raw numbers suggested, and our least urban site (Pond 1) was estimated to be even more male-biased. Furthermore, in using estimated proportions rather than sampled proportions, an additional six fixed effects not significant in raw number models were significant. This emphasizes how sex-specific capture probabilities and thus the reliability of raw estimates may not be constant across sites or across varying levels of effort, even within the same species and geographic area. Although the confidence of our population size estimates vary (Table 3.5), these comparisons emphasize how observed numbers of individuals from sites that are not rigorously sampled should be used with caution in analyses. Only our most frequently sampled site (Pond 4), with a total of 948 trap nights (Table 3.2), had raw numbers of males and females that were very close to population size estimates (Table 3.5). Sites with a lower effort of sampling had population size estimates that differed from sampled numbers more substantially. The minimum effort per site in our study was 41 trap nights (Table 3.2), but previous studies have had as few as 2 trap nights per site (Table 3.1). We urge future researchers to design studies with mark-recapture models in mind, and provide those estimates when considering sex ratios in relation to urbanization. In summation, sex biases may be more extreme than simple raw numbers from trapping data suggest, and these differences may lead to differing conclusions that can be drawn from analyses.

One of the caveats that should be mentioned in our dataset is the difference in recapture percentages across ponds. For instance, Pond 2 is our largest pond in the dataset, with likely a very large population of turtles. However, this site was added a few years into the study, and we were not able to recapture any turtles between 2023 and 2024, precluding us from obtaining mark recapture estimates. Assuring there is similar effort across sites in future studies will increase confidence in population size estimates. Additionally, future studies should aim for replication at each level of urbanization to avoid issues such as the discrepancies we observed in housing density differences in our two most urban sites. Another consideration that should be made when interpreting our findings is that it is possible the urbanization gradient we sampled was not broad enough to detect consistent differences in sex ratios related to urbanization that may be evident in studies that encompass a broader geographic area and range of urbanization levels.

Future studies should emphasize collecting data in a way that allows for accurate mark recapture estimates, involving frequent intensive sampling at regular intervals at each site. Future work would also benefit from investigating temperatures at nests surrounding each site in addition to sex ratios at those sites. Additionally, complementary road surveys to document road mortality could strengthen findings. Conducting trapping during as much of the year as is possible will improve estimates, as males are more mobile more during the breeding season and females are more mobile during the nesting season (Morreale et al., 1984).

There are many negative implications associated with altered sex ratios. A decrease in effective population size likely leads to decreased genetic diversity and thus, decreased adaptive potential in the face of further anthropogenic change. There may also be energetic consequences associated with mate searching. Male-biased populations may increase energetic demands on males seeking females, while females may experience an increase in harassment from competing males. Mitigation measures such as the use of drift fences (Aresco, 2005) or highway underpasses would greatly aid in reducing road mortality. Additionally, the creation of green spaces surrounding ponds in urban areas could better support local turtle populations and reduce female mortality by providing nearby nesting habitat.

Our study adds to the body of work that provides evidence for negative impacts to turtle populations in relation to urbanization, and addresses knowledge gaps on a species and region that are relatively understudied for this topic. Although we present our findings on a species that is not of conservation concern specifically, turtles are one of the most imperiled vertebrate taxa (Lovich et al., 2018) and we must continue to address the constantly emerging threats these animals encounter. Not only do our results provide more empirical data on effects of urbanization on turtle sex ratios, but we show evidence that the use of population size estimates in analyses can greatly impact conclusions drawn from population studies.

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Conclusions

In my dissertation, I investigated various human impacts on turtles at different life stages, from nest site choice, to the embryo and hatchling stages, to sex ratios in adults. Altogether, my findings both emphasize the sensitivity of turtles to various anthropogenic impacts as well as highlight the need for conservation strategies to mitigate these risks.

In Chapter 1, I provided evidence that emydid turtles select specific microhabitat features for their nests, but that they do not adjust their nest site choice in areas with higher levels of human disturbance. The site a female chooses to place her nest influences various hatchling phenotypes as well as survival, highlighting the importance of documenting our impacts on this critical behavior. Although there are a variety of studies characterizing nest site choice in reptiles, few have directly assessed nest site choice in relation to varying levels of urbanization, so this work presents a novel question that lays the groundwork for future research. Although I found that turtles did not alter their nesting strategy in the most urban environments, whether turtles are capable of adjusting their nesting strategy in areas with higher levels of urbanization than my study sites warrants future attention. In this study, I concentrated nest searching effort around the perimeter of aquatic habitats, but future studies could benefit from using telemetry to track nesting females who may make further movements outside of typical search areas. Additionally, further research linking female nest site choice in urban areas to embryo and hatchling survival would be particularly informative.

In Chapter 2, I revealed how multiple conditions during early development, specifically incubation temperature during the embryo stage, and overwintering environment during the hatchling stage, interactively affect hatchling righting performance. The reasons why most hatchling pond sliders overwinter in the nest and emerge in the spring, while some emerge in the fall and overwinter in water, is poorly understood, and few studies have investigated hatchling performance in relation to this life history stage. In this chapter, I took a novel approach in assessing how conditions during multiple developmental stages can influence hatchling performance, which has important implications for survival and ultimately population persistence. I found that the effect of the overwintering environment was dependent upon incubation temperature. Furthermore, evidence suggests that warmer conditions at both developmental stages may potentially have deleterious effects. Aquatically overwintering hatchlings that were incubated at warmer temperatures, and hatched earlier overall, thus spending more time in the warm aquatic environment, were those that performed most poorly in righting response trials. Altogether, results from this study highlight how alterations to climate that affect thermal conditions of nest and overwintering sites may have impacts not only on phenotypes at hatching, but lasting effects that influence performance, and potentially survival. Future studies would benefit from assessing hatchling righting response in both the fall and spring, and replicating natural thermal fluctuations in both terrestrial and aquatic environments. Further investigation into what drives variation in emergence timing in sliders could provide insight into whether and how changes in climate could impact overwintering choices hatchlings make, and the consequences of those choices.

In Chapter 3, I examined the relationship between adult sex ratios and urbanization, and also compared analyses using sampled proportions of males and females versus those that used capture-mark-recapture estimates. Although the topic of urbanization and sex ratios in turtles has been well studied, I introduced a new methodology for the field by employing these capture-mark-recapture methods in an attempt to improve future inferences. While my results showed that the proportion of males in the population was not consistently associated with higher levels of urbanization, I show evidence of a strong male bias at one of the most urban sites sampled, suggesting that female-biased road mortality could be occurring in this population. Additionally, I discuss how differences in landcover, particularly those related to greenspace in residential areas, may be driving the female bias observed at another urban site, due to a reduction in the need for females to travel across multiple roads to nest. Overall, this chapter contributes to a growing body of work in improving our knowledge of the complicated dynamics of turtle sex ratios and urbanization, using an understudied species in an understudied region for the topic. Future researchers should aim to design studies that sample frequently enough to allow for precise capture-mark-recapture estimates. Additionally, studies that document road mortality and local nest temperatures in conjunction with population sampling may clarify whether the urban heat-island effect or female-biased road mortality is more influential in driving sex ratios in urban areas.

In addition to the insights my dissertation chapters provide in terms of responses to human impacts, several of my results emphasize that relying on broad generalizations should be avoided in the field of urban turtle ecology. For instance, I found higher nest temperatures and higher nest predation rates on experimental nests at the least urban site, and a female-biased population at the most urban site sampled, all of which are the opposite of what is generally expected in trends associated with urbanization. Without putting these results into context, one may make the incorrect assumption that anthropogenic impacts are not a driving factor in this system. However, when factors such as differences in nesting substrates, abundances of nests, and urban landcover types are considered, we see that the answer is not always so simple. Future work would benefit from carefully considering unique variables in each system and their effects on population responses.

Collectively, the work in my dissertation furthers our understanding of how human disturbance impacts turtles in different life stages, providing important information for predicting how turtles will persist in anthropogenically altered environments, as well as informing management decisions. Results from all three chapters provide evidence that preserving or creating large areas of greenspace with moderate canopy cover surrounding urban aquatic environments could dramatically improve outcomes for urban turtle populations. This management strategy would be beneficial in a number of ways. As supported by my first chapter, this should preserve natural microhabitat features available for nesting females. As supported by my second chapter, this management strategy could aid in maintaining appropriate thermal conditions for nesting and overwintering sites, preventing the harmful effects of warming in these two developmental environments. And lastly, as suggested in my third chapter, providing a large area with suitable nesting sites surrounding an aquatic habitat may prevent the need for females to travel across roadways and other barriers and thereby reduce female-biased road mortality. It is of utmost importance that we continue to investigate all possible management strategies to mitigate the ever-increasing threats the growing human population presents to wildlife populations, especially those as vulnerable as turtles. It is my hope that the findings in this dissertation can contribute to advancements in the field of turtle conservation, which is, along with a love for turtles, the most motivating factor in all my work.

References

Albecker, M. A., Strobel, S. M., & Womack, M. C. (2023). Developmental Plasticity in Anurans: Meta-analysis Reveals Effects of Larval Environments on Size at Metamorphosis And Timing of Metamorphosis. *Integrative And Comparative Biology*, *63*(3), 714–729. https://doi.org/10.1093/icb/icad059

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, *114*(34), 8951–8956. https://doi.org/10.1073/pnas.1606034114

Ancona, S., Dénes, F. V., Krüger, O., Székely, T., & Beissinger, S. R. (2017). Estimating adult sex ratios in nature. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1729), 20160313. https://doi.org/10.1098/rstb.2016.0313

Anđelković, M., & Bogdanović, N. (2022). Amphibian and Reptile Road Mortality in Special Nature Reserve Obedska Bara, Serbia. *Animals*, *12*(5), Article 5. https://doi.org/10.3390/ani12050561

Andrén, H. (1997). Habitat Fragmentation and Changes in Biodiversity. *Ecological Bulletins*, (46), 171–181.

Angilletta Jr., M. J., Winters, R. S., & Dunham, A. E. (2000). Thermal Effects on the Energetics of Lizard Embryos: Implications for Hatchling Phenotypes. *Ecology*, *81*(11), 2957–2968. https://doi.org/10.1890/0012-9658(2000)081%5B2957:TEOTEO%5D2.0.CO;2

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press.

Aresco, M. J. (2005). Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. *Journal of Wildlife Management*, *69*(2), 549–560. https://doi.org/10.2193/0022-541X(2005)069%5B0549:MMTRHM%5D2.0.CO;2

Arnfield, A. J. (2003). Two decades of urban climate research: A review of turbulence, exchanges of energy and water, and the urban heat island. *International Journal of Climatology*, *23*(1), 1–26. https://doi.org/10.1002/joc.859

Arnold, S. J. (1983). Morphology, Performance and Fitness. *American Zoologist*, *23*(2), 347–361.

Auge, A.-C., Blouin-Demers, G., Hasler, C. T., & Murray, D. L. (2024). Demographic evidence that development is not compatible with sustainability in semi-urban freshwater turtles. *Animal Conservation*, *27*(2), 253–266. https://doi.org/10.1111/acv.12903

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Benard, M. F., & McCauley, S. J. (2008). Integrating across life-history stages: Consequences of natal habitat effects on dispersal. *The American Naturalist*, *171*(5), 553–567.

Ben-Ezra, N., & Burness, G. (2017). Constant and Cycling Incubation Temperatures Have Long-Term Effects on the Morphology and Metabolic Rate of Japanese Quail. *Physiological and Biochemical Zoology*, *90*(1), 96–105. https://doi.org/10.1086/688383

Bennett, V. J. (2017). Effects of Road Density and Pattern on the Conservation of Species and Biodiversity. *Current Landscape Ecology Reports*, *2*(1), 1–11. https://doi.org/10.1007/s40823-017-0020-6

Bodensteiner, B. L., Mitchell, T. S., Strickland, J. T., & Janzen, F. J. (2015). Hydric conditions during incubation influence phenotypes of neonatal reptiles in the field. *Functional Ecology*, *29*(5), 710–717. https://doi.org/10.1111/1365-2435.12382

Bodie, J. R., & Semlitsch, R. D. (2000). Spatial and temporal use of floodplain habitats by lentic and lotic species of aquatic turtles. *Oecologia*, *122*(1), 138–146. https://doi.org/10.1007/PL00008830

Booth, D. T. (2006). Influence of Incubation Temperature on Hatchling Phenotype in Reptiles. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *79*(2), 274–281. (edsjsr.10.1086.499988). https://doi.org/10.1086/499988

Booth, D. T., Thompson, M. B., & Herring, S. (2000). How incubation temperature influences the physiology and growth of embryonic lizards. *Journal of*

Comparative Physiology B, *170*(4), 269–276. https://doi.org/10.1007/s003600000097

Borges, F. J. A., & Marini, M. Â. (2010). Birds nesting survival in disturbed and protected Neotropical savannas. *Biodiversity and Conservation*, *19*(1), 223–236. https://doi.org/10.1007/s10531-009-9718-z

Bowen, William. D., den Heyer, C. E., McMillan, J. I., & Iverson, S. J. (2015). Offspring size at weaning affects survival to recruitment and reproductive performance of primiparous gray seals. *Ecology and Evolution*, *5*(7), 1412–1424. https://doi.org/10.1002/ece3.1450

Bowne, D. R., Cosentino, B. J., Anderson, L. J., Bloch, C. P., Cooke, S., Crumrine, P. W., Dallas, J., Doran, A., Dosch, J. J., Druckenbrod, D. L., Durtsche, R. D., Garneau, D., Genet, K. S., Fredericksen, T. S., Kish, P. A., Kolozsvary, M. B., Kuserk, F. T., Lindquist, E. S., Mankiewicz, C., … Zimmermann, C. R. (2018). Effects of urbanization on the population structure of freshwater turtles across the United States. *Conservation Biology*, *32*(5), 1150–1161. https://doi.org/10.1111/cobi.13136

Breitenbach, G. L., Congdon, J. D., & Sels, R. C. van L. (1984). Winter Temperatures of *Chrysemys picta* Nests in Michigan: Effects on Hatchling Survival. *Herpetologica*, *40*(1), 76–81.

Brischoux, F., Dupoué, A., Lourdais, O., & Angelier, F. (2016). Effects of mild wintering conditions on body mass and corticosterone levels in a temperate reptile, the aspic viper (*Vipera aspis*). *Comparative Biochemistry and Physiology Part A:*

Molecular & Integrative Physiology, *192*, 52–56. https://doi.org/10.1016/j.cbpa.2015.11.015

Brown, C. R., & Bomberger Brown, M. (2013a). Where has all the road kill gone? *Current Biology*, *23*(6), R233–R234. https://doi.org/10.1016/j.cub.2013.02.023

Brown, C. R., & Bomberger Brown, M. (2013b). Where has all the road kill gone? *Current Biology*, *23*(6), R233–R234. https://doi.org/10.1016/j.cub.2013.02.023

Brown, G. P., & Shine, R. (2004). Maternal Nest-Site Choice and Offspring Fitness in a Tropical Snake (*Tropidonophis mairii*, Colubridae). *Ecology*, *85*(6), 1627–1634. https://doi.org/10.1890/03-0107

Buhlmann, K. A., Akre, T. S. B., Iverson, J. B., Karapatakis, D., Mittermeier, R. A., Georges, A., Rhodin, A. G. J., Dijk, P. P. van, & Gibbons, J. W. (2009). A Global Analysis of Tortoise and Freshwater Turtle Distributions with Identification of Priority Conservation Areas. *Chelonian Conservation and Biology*, *8*(2), 116–149. https://doi.org/10.2744/CCB-0774.1

Buhlmann, K. A., & Coffman, G. (2001). Fire Ant Predation of Turtle Nests and Implications for the Strategy of Delayed Emergence. *Journal of the Elisha Mitchell Scientific Society*, *117*(2), 94–100.

Burnham, K. P., & Anderson, D. R. (Eds.). (2002). Advanced Issues and Deeper Insights. In *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (pp. 267–351). Springer. https://doi.org/10.1007/978-0-387-22456-5_6

Butler, D. G., Cullis, B. R., Gilmour, A. R., & Gogel, B. J. (2009). *Asreml: Asreml() fits the linear mixed model. R package version 3.0. Www.vsni.co.uk.* [Computer software].

Buxton, V. L., Santymire, R. M., & Benson, T. J. (2018). Mixed effects of urbanization on density, nest survival, and nestling corticosterone of a generalist passerine. *Ecosphere*, *9*(12), e02517. https://doi.org/10.1002/ecs2.2517

Buzuleciu, S. A., Crane, D. P., & Parker, S. L. (2016). Scent of Disinterred Soil as an Olfactory Cue used by Raccoons to Locate Nests of Diamond-backed Terrapins. *Herpetological Conservation and Biology*, *11*, 539–551.

Cagle, F. R. (1939). A System of Marking Turtles for Future Identification. *Copeia*, *1939*(3), 170–173. https://doi.org/10.2307/1436818

Cagle, F. R. (1944). Sexual Maturity in the Female of the Turtle *Pseudemys scripta* elegans. *Copeia*, *1944*(3), 149–152. https://doi.org/10.2307/1437808

Cagle, F. R. (1948). Sexual maturity in the male turtle, *Pseudemys scripta troostii*. *Copeia*, *1948*(2), 108–111.

Cagle, K. D., Packard, G. C., Miller, K., & Packard, M. J. (1993). Effects of the Microclimate in Natural Nests on Development of Embryonic Painted Turtles, *Chrysemys picta*. *Functional Ecology*, *7*(6), 653–660. https://doi.org/10.2307/2390185

Cam, E., Monnat, J.-Y., & Hines, J. E. (2003). Long-term fitness consequences of early conditions in the kittiwake. *Journal of Animal Ecology*, *72*(3), 411–424. https://doi.org/10.1046/j.1365-2656.2003.00708.x

Carter, A. W., Paitz, R. T., McGhee, K. E., & Bowden, R. M. (2016). Turtle hatchlings show behavioral types that are robust to developmental manipulations. *Physiology & Behavior*, *155*, 46–55. https://doi.org/10.1016/j.physbeh.2015.11.034

Casale, P., Freggi, D., Furii, G., Vallini, C., Salvemini, P., Deflorio, M., Totaro, G., Raimondi, S., Fortuna, C., & Godley, B. J. (2015). Annual survival probabilities of juvenile loggerhead sea turtles indicate high anthropogenic impact on Mediterranean populations. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *25*(5), 690–700.

Chovanec, A. (1994). Man-made wetlands in urban recreational areas—A habitat for endangered species? *Landscape and Urban Planning*, *29*(1), 43–54. https://doi.org/10.1016/0169-2046(93)00015-H

Christian, K. A., Tracy, C. R., & Porter, W. P. (1986). The Effect of Cold Exposure during Incubation of Sceloporus undulatus Eggs. *Copeia*, *1986*(4), 1012–1014. https://doi.org/10.2307/1445303

Collins, M. K., Magle, S. B., & Gallo, T. (2021). Global trends in urban wildlife ecology and conservation. *Biological Conservation*, *261*, 109236. https://doi.org/10.1016/j.biocon.2021.109236

Congdon, J. D., Breitenbach, G. L., Sels, R. C. van L., & Tinkle, D. W. (1987). Reproduction and Nesting Ecology of Snapping Turtles (*Chelydra serpentina*) in Southeastern Michigan. *Herpetologica*, *43*(1), 39–54.

Congdon, J. D., Dunham, A. E., & Sels, R. C. V. L. (1993). Delayed Sexual Maturity and Demographics of Blanding’s Turtles (*Emydoidea blandingii*): Implications for

Conservation and Management of Long-Lived Organisms. *Conservation Biology*, *7*(4), 826–833.

Congdon, J. D., Tinkle, D. W., Breitenbach, G. L., & Sels, R. C. van L. (1983). Nesting Ecology and Hatching Success in the Turtle *Emydoidea blandingi*. *Herpetologica*, *39*(4), 417–429.

Cordonnier, M., Gibert, C., Bellec, A., Kaufmann, B., & Escarguel, G. (2019). Multi-scale impacts of urbanization on species distribution within the genus Tetramorium. *Landscape Ecology*, *34*(8), 1937–1948. https://doi.org/10.1007/s10980-019-00842-7

Costanzo, J. P., Lee JR, R. E., & Ultsch, G. R. (2008). Physiological ecology of overwintering in hatchling turtles. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *309A*(6), 297–379. https://doi.org/10.1002/jez.460

Courchamp, F., Clutton-Brock, T., & Grenfell, B. (1999). Inverse density dependence and the Allee effect. *Trends in Ecology & Evolution*, *14*(10), 405–410. https://doi.org/10.1016/S0169-5347(99)01683-3

Czech, B., Krausman, P. R., & Devers, P. K. (2000). Economic Associations among Causes of Species Endangerment in the United States: Associations among causes of species endangerment in the United States reflect the integration of economic sectors, supporting the theory and evidence that economic growth proceeds at the competitive exclusion of nonhuman species in the aggregate. *BioScience*, *50*(7), 593–601. https://doi.org/10.1641/0006-3568(2000)050%5B0593:EAACOS%5D2.0.CO;2

Dalgish, J., & Anderson, S. (1979). A Field Experiment on Learning by Raccoons. *Journal of Mammalogy*, *60*(3), 620–622. https://doi.org/10.2307/1380105

Davy, C. M., Paterson, J. E., & Leifso, A. E. (2014). When righting is wrong: Performance measures require rank repeatability for estimates of individual fitness. *Animal Behaviour*, *93*, 15–23. https://doi.org/10.1016/j.anbehav.2014.04.013

Dawson, S. J., Adams, P. J., Huston, R. M., & Fleming, P. A. (2014). Environmental factors influence nest excavation by foxes. *Journal of Zoology*, *294*(2), 104–113. https://doi.org/10.1111/jzo.12158

de Jong, M., Phillips, B. L., Llewelyn, J., Chapple, D. G., & Wong, B. B. M. (2022). Effects of developmental environment on animal personality in a tropical skink. *Behavioral Ecology and Sociobiology*, *76*(10), 137. https://doi.org/10.1007/s00265-022-03240-3

DeCatanzaro, R., & Chow-Fraser, P. (2010). Relationship of road density and marsh condition to turtle assemblage characteristics in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *36*(2), 357–365. https://doi.org/10.1016/j.jglr.2010.02.003

Delmas, V., Baudry, E., Girondot, M., & Prevot-Julliard, A. (2007). The righting response as a fitness index in freshwater turtles. *Biological Journal of the Linnean Society*, *91*(1), 99–109. https://doi.org/10.1111/j.1095-8312.2007.00780.x

Domokos, G., & Várkonyi, P. L. (2007). Geometry and self-righting of turtles. *Proceedings of the Royal Society B: Biological Sciences*. (London). https://doi.org/10.1098/rspb.2007.1188

Doody, J. S., Guarino, E. N. Z. O., Harlow, P., & Corey, B. (2006). Quantifying Nest Site Choice in Reptiles Using Hemispherical Photography and Gap Light Analysis. *Herpetological Review*, *37*(1), 49–51.

Dorland, A., Rytwinski, T., & Fahrig, L. (2014). Do Roads Reduce Painted Turtle (*Chrysemys picta*) Populations? *PLOS ONE*, *9*(5), e98414. https://doi.org/10.1371/journal.pone.0098414

Downing, R. J., Rytwinski, T., & Fahrig, L. (2015). Positive effects of roads on small mammals: A test of the predation release hypothesis. *Ecological Research*, *30*(4), 651–662. https://doi.org/10.1007/s11284-015-1264-4

Du, W.-G., & Shine, R. (2015). The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. *Biological Reviews*, *90*(1), 19–30. https://doi.org/10.1111/brv.12089

Dupuis-Désormeaux, M., D’Elia, V., Burns, R., White, B., & MacDonald, S. E. (2019). A turtle population study in an isolated urban wetland complex in Ontario reveals a few surprises. *FACETS*, *4*(1), 584–597. https://doi.org/10.1139/facets-2019-0046

DuRant, S. E., Hopkins, W. A., Wilson, A. F., & Hepp, G. R. (2012). Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird. *Functional Ecology*, *26*(2), 416–422. https://doi.org/10.1111/j.1365-2435.2011.01945.x

Elnitsky, M. A., & Claussen, D. L. (2006). The effects of temperature and inter-individual variation on the locomotor performance of juvenile turtles. *Journal of Comparative Physiology B*, *176*(6), 497–504. https://doi.org/10.1007/s00360-006-0071-1

Eötvös, C. B., Magura, T., & Lövei, G. L. (2018). A meta-analysis indicates reduced predation pressure with increasing urbanization. *Landscape and Urban Planning*, *180*, 54–59. https://doi.org/10.1016/j.landurbplan.2018.08.010

Ernst, C. H. (1972). Temperature-activity relationship in the painted turtle, *Chrysemys picta*. *Copeia*, 217–222.

Ernst, C. H., & Lovich, J. E. (2009). *Turtles of the United States and Canada*. The John Hopkins University Press.

Ewert, M. A. (1985). *Embryology of turtles. In "Biology of the Reptilia." (Eds C Gans, F Billett, PFA Manderson.)* (Vol. 14). John Wiley & Sons: New York.

Ewert, M. A., & Nelson, C. E. (1991). Sex Determination in Turtles: Diverse Patterns and Some Possible Adaptive Values. *Copeia*, *1991*(1), 50–69. https://doi.org/10.2307/1446248

Faulkner, S. (2004). Urbanization impacts on the structure and function of forested wetlands. *Urban Ecosystems*, *7*(2), 89–106. https://doi.org/10.1023/B:UECO.0000036269.56249.66

Feinberg, J. A., & Burke, R. L. (2003a). Nesting Ecology and Predation of Diamondback Terrapins, *Malaclemys terrapin*, at Gateway National Recreation Area, New York. *Journal of Herpetology*, *37*(3), 517–526.

Feinberg, J. A., & Burke, R. L. (2003b). Nesting Ecology and Predation of Diamondback Terrapins, *Malaclemys terrapin*, at Gateway National Recreation Area, New York. *Journal of Herpetology*, *37*(3), 517–526. https://doi.org/10.1670/207-02A

Fisher, L. R., Godfrey, M. H., & Owens, D. W. (2014). Incubation Temperature Effects on Hatchling Performance in the Loggerhead Sea Turtle (*Caretta caretta*). *PLoS ONE*, *9*(12), e114880. https://doi.org/10.1371/journal.pone.0114880

Foley, S. M., Price, S. J., & Dorcas, M. E. (2012). Nest-site selection and nest depredation of semi-aquatic turtles on golf courses. *Urban Ecosystems*, *15*(2), 489–497. https://doi.org/10.1007/s11252-012-0229-4

Folkerts Caldwell, M., López-Pérez, J. E., Warner, D. A., & Wolak, M. E. (2023). Consistent Nest Site Selection by Turtles across Habitats with Varying Levels of Human Disturbance. *Diversity*, *15*(2), 275. https://doi.org/10.3390/d15020275

Folkerts Caldwell, M., Warner, D. A., & Wolak, M. E. (2026). Incubation and Overwintering Conditions Influence Righting Performance of Hatchling Turtles. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, jez.70065. https://doi.org/10.1002/jez.70065

Freeman, E. D., Larsen, R. T., Peterson, M. E., Anderson Jr., C. R., Hersey, K. R., & Mcmillan, B. R. (2014). Effects of male-biased harvest on mule deer: Implications for rates of pregnancy, synchrony, and timing of parturition. *Wildlife Society Bulletin*, *38*(4), 806–811. https://doi.org/10.1002/wsb.450

Georges, A., Beggs, K., Young, J. E., & Doody, J. S. (2005). Modelling Development of Reptile Embryos under Fluctuating Temperature Regimes. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *78*(1), 18–30. https://doi.org/10.1086/425200

Geslin, B., Le Féon, V., Folschweiller, M., Flacher, F., Carmignac, D., Motard, E., Perret, S., & Dajoz, I. (2016). The proportion of impervious surfaces at the landscape scale structures wild bee assemblages in a densely populated region. *Ecology and Evolution*, *6*(18), 6599–6615. https://doi.org/10.1002/ece3.2374

Gibbons, J. W. (1970). Terrestrial Activity and the Population Dynamics of Aquatic Turtles. *The American Midland Naturalist*, *83*(2), 404–414. https://doi.org/10.2307/2423953

Gibbons, J. W. (1986). Movement Patterns among Turtle Populations: Applicability to Management of the Desert Tortoise. *Herpetologica*, *42*(1), 104–113.

Gibbons, J. W. (1990). *Life History and Ecology of the Slider Turtle*. Smithsonian Institution Press.

Gibbons, J. W. (2013). A Long-Term Perspective of Delayed Emergence (aka Overwintering) in Hatchling Turtles: Some They Do and Some They Don’t, and Some You Just Can’t Tell. *Journal of Herpetology*, *47*(2), 203–214. https://doi.org/10.1670/12-122

Gibbs, J. P., & Steen, D. A. (2005). Trends in Sex Ratios of Turtles in the United States: Implications of Road Mortality. *Conservation Biology*, *19*(2), 552–556. https://doi.org/10.1111/j.1523-1739.2005.000155.x

Gilbert, A. L., Wayne, S. M., Norris, M. C., Rodgers, J. M., & Warner, D. A. (2024). Stressful Body Temperatures as a Maternal Effect on Lizard Reproduction. *Ecological and Evolutionary Physiology*, *97*(5), 292–301.

Guyer, C., Bailey, M. A., & Mount, R. H. (2015). *Turtles of Alabama*. University of Alabama Press.

Habib, L., Bayne, E. M., & Boutin, S. (2007). Chronic industrial noise affects pairing success and age structure of ovenbirds Seiurus aurocapilla. *Journal of Applied Ecology*, *44*(1), 176–184. https://doi.org/10.1111/j.1365-2664.2006.01234.x

Hartig, F. (2024). *_DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models_* [R package version 0.4.7]. https://CRAN.R-project.org/package=DHARMa

Heppell, S. S. (1998). Application of life-history theory and population model analysis to turtle conservation. *Copeia*, 367–375.

Hill, R. A., Weber, M. H., Leibowitz, S. G., Olsen, A. R., & Thornbrugh, D. J. (2016). The Stream-Catchment (StreamCat) Dataset: A Database of Watershed Metrics for the Conterminous United States. *JAWRA Journal of the American Water Resources Association*, *52*(1), 120–128. https://doi.org/10.1111/1752-1688.12372

Hoffman, A. J., Dees, L., & Wada, H. (2023). Heat-induced maternal effects shape avian eggshell traits and embryo development and phenotype at high incubation temperatures. *Ecology and Evolution*, *13*(9), e10546. https://doi.org/10.1002/ece3.10546

Holcomb, S. R., & Carr, J. L. (2013). Mammalian Depredation of Artificial Alligator Snapping Turtle (*Macrochelys temminckii*) Nests in North Louisiana. *Southeastern Naturalist*, *12*(3), 478–491. https://doi.org/10.1656/058.012.0303

Hope, S. F., Hopkins, W. A., & Angelier, F. (2022). Parenting in the city: Effects of urbanization on incubation behaviour and egg temperature in great tits, Parus major. *Animal Behaviour*, *194*, 1–11. https://doi.org/10.1016/j.anbehav.2022.09.004

Inouye, D. W. (2022). Climate change and phenology. *WIREs Climate Change*, *13*(3), e764. https://doi.org/10.1002/wcc.764

Isaksson, C. (2015). Urbanization, oxidative stress and inflammation: A question of evolving, acclimatizing or coping with urban environmental stress. *Functional Ecology*, *29*(7), 913–923. https://doi.org/10.1111/1365-2435.12477

Jackson, C. G. (1970). A Biometrical Study of Growth in Pseudemys concinna suwanniensis. I. *Copeia*, *1970*(3), 528–534. https://doi.org/10.2307/1442280

Jackson, N., Cristescu, R. H., Piza-Roca, C., Littleford-Colquhoun, B. L., Strickland, K., & Frère, C. H. (2019). Maternal nesting behaviour in city dragons: A species with temperature-dependent sex determination. *Journal of Urban Ecology*, *5*(1), juz005. https://doi.org/10.1093/jue/juz005

James Reynolds, S., Ibáñez-Álamo, J. D., Sumasgutner, P., & Mainwaring, M. C. (2019). Urbanisation and nest building in birds: A review of threats and opportunities. *Journal of Ornithology*, *160*(3), 841–860. https://doi.org/10.1007/s10336-019-01657-8

Janzen, F. J. (1993). An Experimental Analysis of Natural Selection on Body Size of Hatchling Turtles. *Ecology*, *74*(2), 332–341. https://doi.org/10.2307/1939296

Janzen, F. J. (1994a). Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences*, *91*(16), 7487–7490. https://doi.org/10.1073/pnas.91.16.7487

Janzen, F. J. (1994b). Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences*, *91*(16), 7487–7490. https://doi.org/10.1073/pnas.91.16.7487

Janzen, F. J., & Morjan, C. L. (2001). Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour*, *62*(1), 73–82. https://doi.org/10.1006/anbe.2000.1732

Janzen, F. J., Tucker, J. K., & Paukstis, G. L. (2000). Experimental Analysis of an Early Life-History Stage: Selection on Size of Hatchling Turtles. *Ecology*, *81*(8), 2290–2304. https://doi.org/10.1890/0012-9658(2000)081%5B2290:EAOAEL%5D2.0.CO;2

Johnson, W. C., & Collinge, S. K. (2004). Landscape effects on black-tailed prairie dog colonies. *Biological Conservation*, *115*(3), 487–497. https://doi.org/10.1016/S0006-3207(03)00165-4

Jokimäki, J., & Huhta, E. (2000). Artifical nest predation and abundance of birds along an urban gradient. *Condor*, (102), 838–847.

Jolly, G. M. (1965). Explicit Estimates from Capture-Recapture Data with Both Death and Immigration-Stochastic Model. *Biometrika*, *52*(1/2), 225–247. https://doi.org/10.2307/2333826

Kemp, S. J. (2022). Decline of Woodland Box Turtles (*Terrapene carolina carolina*) over 40 Years in Southeastern Pennsylvania, USA. *Herpetological Conservation and Biology*, *17*(1), 196–203.

Kleewein, A. (2015). Investigating temperature tolerance in wild broods of *Trachemys scripta elegans* (Reptilia: Testudines: Emydidae) in Austria. *Hyla: Herpetological bulletin*, *2015*(1), 28–35.

Kolbe, J. J., & Janzen, F. J. (2002). Impact of Nest-Site Selection on Nest Success and Nest Temperature in Natural and Disturbed Habitats. *Ecology*, *83*(1), 269–281. https://doi.org/10.1890/0012-9658(2002)083%5B0269:IONSSO%5D2.0.CO;2

Kretser, H. E., Sullivan, P. J., & Knuth, B. A. (2008). Housing density as an indicator of spatial patterns of reported human–wildlife interactions in Northern New York. *Landscape and Urban Planning*, *84*(3), 282–292. https://doi.org/10.1016/j.landurbplan.2007.08.007

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.

Laake, J. L. (2013). *RMark: An R Interface for Analysis of Capture-Recapture Data with MARK*. 1–25.

Lambert, M. R., & Steen, D. A. (2019). Reexamining effects of urbanization on population structure of freshwater turtles: Response to Bowne et al. 2018. *Conservation Biology*, *33*(5), 1193–1196.

Le Galliard, J.-F., Cote, J., & Fitze, P. S. (2008). Lifetime and Intergenerational Fitness Consequences of Harmful Male Interactions for Female Lizards. *Ecology*, *89*(1), 56–64. https://doi.org/10.1890/06-2076.1

Lehtonen, T. K., Babic, N. L., Piepponen, T., Valkeeniemi, O., Borshagovski, A.-M., & Kaitala, A. (2021). High road mortality during female-biased larval dispersal in an iconic beetle. *Behavioral Ecology and Sociobiology*, *75*(1), 26. https://doi.org/10.1007/s00265-020-02962-6

Leighton, P. A., Horrocks, J. A., & Kramer, D. L. (2010). Conservation and the scarecrow effect: Can human activity benefit threatened species by displacing predators? *Biological Conservation*, *143*(9), 2156–2163. https://doi.org/10.1016/j.biocon.2010.05.028

Les, H. L., Paitz, R. T., & Bowden, R. M. (2009). Living at Extremes: Development at the Edges of Viable Temperature under Constant and Fluctuating Conditions. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *82*(2), 105–112. https://doi.org/10.1086/590263

Lewis, E. L., Iverson, J. B., Smith, G. R., & Rettig, J. E. (2018). Body size and growth in the red-eared slider. *Herpetological Conservation and Biology*, *13*(3), 700–710.

Ligon, D. B., Peterson, C. C., & Lovern, M. B. (2012). Acute and Persistent Effects of Pre- and Posthatching Thermal Environments on Growth and Metabolism in the Red-Eared Slider Turtle, *Trachemys scripta elegans*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *317*(4), 227–235. https://doi.org/10.1002/jez.1716

Lind, M. I., & Johansson, F. (2007). The degree of adaptive phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. *Journal of Evolutionary Biology*, *20*(4), 1288–1297. https://doi.org/10.1111/j.1420-9101.2007.01353.x

Lloyd, R. B., & Warner, D. A. (2019). Maternal nest-site choice does not affect egg hatching success in an invasive turtle population. *Behaviour*, *156*(3–4), 265–285. https://doi.org/10.1163/1568539X-00003541

Lovich, J. E., Ennen, J. R., Agha, M., & Gibbons, J. W. (2018). Where Have All the Turtles Gone, and Why Does It Matter? *BioScience*, *68*(10), 771–781. https://doi.org/10.1093/biosci/biy095

Madsen, T., & Shine, R. (1999). Life History Consequences of Nest-Site Variation in Tropical Pythons (*Liasis fuscus*). *Ecology*, *80*(3), 989–997. https://doi.org/10.1890/0012-9658(1999)080%5B0989:LHCONS%5D2.0.CO;2

Marchand, M. N., & Litvaitis, J. A. (2004). Effects of Habitat Features and Landscape Composition on the Population Structure of a Common Aquatic Turtle in a Region Undergoing Rapid Development. *Conservation Biology*, *18*(3), 758–767. https://doi.org/10.1111/j.1523-1739.2004.00019.x

Marchand, M. N., Litvaitis, J. A., Maier, T. J., & DeGraaf, R. M. (2002). Use of Artificial Nests to Investigate Predation on Freshwater Turtle Nests. *Wildlife Society Bulletin (1973-2006)*, *30*(4), 1092–1098.

Marshall, H. H., Vitikainen, E. I. K., Mwanguhya, F., Businge, R., Kyabulima, S., Hares, M. C., Inzani, E., Kalema-Zikusoka, G., Mwesige, K., Nichols, H. J., Sanderson, J. L., Thompson, F. J., & Cant, M. A. (2017). Lifetime fitness consequences of early-life ecological hardship in a wild mammal population. *Ecology and Evolution*, *7*(6), 1712–1724. https://doi.org/10.1002/ece3.2747

Mcdonald, R. I., Kareiva, P., & Forman, R. T. T. (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation.

Biological Conservation, *141*(6), 1695–1703. https://doi.org/10.1016/j.biocon.2008.04.025

McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, *52*(10), 883–890. https://doi.org/10.1641/0006-3568(2002)052%5B0883:UBAC%5D2.0.CO;2

Meyer, K. (2008). Likelihood calculations to evaluate experimental designs to estimate genetic variances. *Heredity*, (101), 212–221.

Micheli-Campbell, M. A., Campbell, H. A., Cramp, R. L., Booth, D. T., & Franklin, C. E. (2011). Staying cool, keeping strong: Incubation temperature affects performance in a freshwater turtle. *Journal of Zoology*, *285*(4), 266–273. https://doi.org/10.1111/j.1469-7998.2011.00840.x

Mitchell, T. S., Janzen, F. J., & Warner, D. A. (2018). Quantifying the effects of embryonic phenotypic plasticity on adult phenotypes in reptiles: A review of current knowledge and major gaps. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 203–214. https://doi.org/10.1002/jez.2187

Mitchell, T. S., Myers, E. M., Tucker, J. K., & McGaugh, S. E. (2017). Righting ability in hatchling turtles does not predict survival during dispersal in the field. *Biological Journal of the Linnean Society*, *120*(3), 553–562.

Mitchell, T. S., Warner, D. A., & Janzen, F. J. (2013). Phenotypic and fitness consequences of maternal nest-site choice across multiple early life stages. *Ecology*, *94*(2), 336–345.

Mittermeier, R. A., van Dijk, P. P., Rhodin, A. G. J., & Nash, S. D. (2015). Turtle Hotspots: An Analysis of the Occurrence of Tortoises and Freshwater Turtles in Biodiversity Hotspots, High-Biodiversity Wilderness Areas, and Turtle Priority Areas. *Chelonian Conservation and Biology*, *14*(1), 2–10. https://doi.org/10.2744/ccab-14-01-2-10.1

Moore, M. J. C., & Seigel, R. A. (2006). No place to nest or bask: Effects of human disturbance on the nesting and basking habits of yellow-blotched map turtles (*Graptemys flavimaculata*). *Biological Conservation*, *130*(3), 386–393. https://doi.org/10.1016/j.biocon.2006.01.001

Morreale, S. J., Gibbons, J. W., & Congdon, J. D. (1984). Significance of activity and movement in the yellow-bellied slider turtle (*Pseudemys scripta*). *Canadian Journal of Zoology*, *62*(6), 1038–1042. https://doi.org/10.1139/z84-148

Muir, T. J., Dishong, B. D., Lee, R. E., & Costanzo, J. P. (2013). Energy use and management of energy reserves in hatchling turtles (*Chrysemys picta*) exposed to variable winter conditions. *Journal of Thermal Biology*, *38*(6), 324–330. https://doi.org/10.1016/j.jtherbio.2013.04.003

Murray, M. H., Becker, D. J., Hall, R. J., & Hernandez, S. M. (2016). Wildlife health and supplemental feeding: A review and management recommendations. *Biological Conservation*, *204*, 163–174. https://doi.org/10.1016/j.biocon.2016.10.034

Muth, A. (1980). Physiological Ecology of Desert Iguana (Dipsosaurus Dorsalis) Eggs: Temperature and Water Relations. *Ecology*, *61*(6), 1335–1343. https://doi.org/10.2307/1939042

Myers, E. M., Tucker, J. K., & Chandler, C. H. (2007). Experimental Analysis of Body Size and Shape during Critical Life-History Events of Hatchling Slider Turtles, *Trachemys scripta elegans*. *Functional Ecology*, *21*(6), 1106–1114.

Najbar, B., & Szuszkiewicz, E. (2007). Nest-site fidelity of the European pond turtle *Emys orbicularis* (Linnaeus, 1758) (Testudines: Emydidae) in western Poland. *Acta Zoologica Cracoviensia - Series A: Vertebrata*, *50*(1–2), 1–8. https://doi.org/10.3409/000000007783995417

Nichols, H., Carter, A. W., Paitz, R. T., & Bowden, R. M. (2019). Red-eared slider hatchlings (*Trachemys scripta*) show a seasonal shift in behavioral types. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *331*(9), 485–493. https://doi.org/10.1002/jez.2315

Noble, D. W., Stenhouse, V., & Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta-analysis. *Biological Reviews*, *93*(1), 72–97.

O’Brien, S., Robert, B., & Tiandry, H. (2005). Consequences of violating the recapture duration assumption of mark–recapture models: A test using simulated and empirical data from an endangered tortoise population. *Journal of Applied Ecology*, *42*(6), 1096–1104. https://doi.org/10.1111/j.1365-2664.2005.01084.x

Ogden, J. C. (1991). Nesting by Wood Storks in Natural, Altered, and Artificial Wetlands in Central and Northern Florida. *Colonial Waterbirds*, *14*(1), 39–45. https://doi.org/10.2307/1521277

Olsson, M., & Madsen, T. (2001). Between-Year Variation in Determinants of Offspring Survival in the Sand Lizard, *Lacerta agilis*. *Functional Ecology*, *15*(4), 443–450.

Orams, M. B. (2002). Feeding wildlife as a tourism attraction: A review of issues and impacts. *Tourism Management*, *23*(3), 281–293. https://doi.org/10.1016/S0261-5177(01)00080-2

Packard, G. C., Packard, M. J., & Birchard, G. F. (1989). Sexual Differentiation and Hatching Success by Painted Turtles Incubating in Different Thermal and Hydric Environments. *Herpetologica*, *45*(4), 385–392.

Packard, G. C., Packard, M. J., Miller, K., & Boardman, T. J. (1987). Influence of Moisture, Temperature, and Substrate on Snapping Turtle Eggs and Embryos. *Ecology*, *68*(4), 983–993. https://doi.org/10.2307/1938369

Parker, W. S. (1984). Immigration and Dispersal of Slider Turtles *Pseudemys scripta* in Mississippi Farm Ponds. *The American Midland Naturalist*, *112*(2), 280–293. https://doi.org/10.2307/2425435

Paterson, J. E., Steinberg, B. D., & Litzgus, J. D. (2014). Effects of body size, habitat selection and exposure on hatchling turtle survival. *Journal of Zoology*, *294*(4), 278–285.

Patrick, D. A., & Gibbs, J. P. (2010). Population structure and movements of freshwater turtles across a road-density gradient. *Landscape Ecology*, *25*(5), 791–801. https://doi.org/10.1007/s10980-010-9459-0

Pauchard, A., Aguayo, M., Peña, E., & Urrutia, R. (2006). Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). *Biological Conservation, Urbanization*, *127*(3), 272–281. https://doi.org/10.1016/j.biocon.2005.05.015

Pelech, S. A., Smith, J. N. M., & Boutin, S. (2010). A predator's perspective of nest predation: Predation by red squirrels is learned, not incidental. *Oikos*, *119*(5), 841–851. https://doi.org/10.1111/j.1600-1706.2009.17786.x

Peng, S., Piao, S., Ciais, P., Friedlingstein, P., Ottle, C., Bréon, F.-M., Nan, H., Zhou, L., & Myneni, R. B. (2012). Surface Urban Heat Island Across 419 Global Big Cities. *Environmental Science & Technology*, *46*(2), 696–703. https://doi.org/10.1021/es2030438

Pottier, P., Burke, S., Zhang, R. Y., Noble, D. W., Schwanz, L. E., Drobniak, S. M., & Nakagawa, S. (2022). Developmental plasticity in thermal tolerance: Ontogenetic variation, persistence, and future directions. *Ecology Letters*, *25*(10), 2245–2268.

Prange, S., Gehrt, S. D., & Wiggers, E. P. (2004). Influences of anthropogenic resources on raccoon (*Procoyon lotor*) movements and spatial distribution. *Journal of Mammalogy*, *85*(3).

Pruett, J. E., Addis, E. A., & Warner, D. A. (2019). The influence of maternal nesting behaviour on offspring survival: Evidence from correlational and cross-fostering studies. *Animal Behaviour*, *153*, 15–24. https://doi.org/10.1016/j.anbehav.2019.04.010

R Core Team. (2022). *R: A language and environment for statistical computing.* [Computer software]. R Foundation for Statistical Computing. https://www.R-project.org/

Rand, A. S., & Dugan, B. (1983). Structure of Complex Iguana Nests. *Copeia*, *1983*(3), 705–711. https://doi.org/10.2307/1444336

Räsänen, K., Laurila, A., & Merilä, J. (2005). Maternal investment in egg size: Environment- and population-specific effects on offspring performance. *Oecologia*, *142*(4), 546–553. https://doi.org/10.1007/s00442-004-1762-5

Ratnaswamy, M. J., Warren, R. J., Kramer, M. T., & Adam, M. D. (1997). Comparisons of Lethal and Nonlethal Techniques to Reduce Raccoon Depredation of Sea Turtle Nests. *The Journal of Wildlife Management*, *61*(2), 368–376. https://doi.org/10.2307/3802593

Reale, J. A., & Blair, R. B. (2005). Nesting Success and Life-History Attributes of Bird Communities Along an Urbanization Gradient. *Urban Habitats*, *3*(1).

Ream, C., & Ream, R. (1966). The Influence of Sampling Methods on the Estimation of Population Structure in Painted Turtles. *The American Midland Naturalist*, *75*(2), 325–338. https://doi.org/10.2307/2423395

Reed, T. E., Waples, R. S., Schindler, D. E., Hard, J. J., & Kinnison, M. T. (2010). Phenotypic plasticity and population viability: The importance of environmental predictability. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1699), 3391–3400.

Refsnider, J. M., Clifton, I. T., & Vazquez, T. K. (2019). Developmental plasticity of thermal ecology traits in reptiles: Trends, potential benefits, and research needs. *Journal of Thermal Biology*, *84*, 74–82.

Refsnider, J. M., & Janzen, F. J. (2010). Putting Eggs in One Basket: Ecological and Evolutionary Hypotheses for Variation in Oviposition-Site Choice. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 39–57.

Refsnider, J. M., Reedy, A. M., Warner, D. A., & Janzen, F. J. (2015). Do trade-offs between predation pressures on females versus nests drive nest-site choice in painted turtles? *Biological Journal of the Linnean Society*, *116*(4), 847–855. https://doi.org/10.1111/bij.12671

Reid, B. N., & Peery, M. Z. (2014). Land use patterns skew sex ratios, decrease genetic diversity and trump the effects of recent climate change in an endangered turtle. *Diversity and Distributions*, *20*(12), 1425–1437. https://doi.org/10.1111/ddi.12243

Reinke, B. A., Miller, D. A. W., & Janzen, F. J. (2019). What Have Long-Term Field Studies Taught Us About Population Dynamics? *Annual Review of Ecology, Evolution, and Systematics*, *50*(1), 261–278. https://doi.org/10.1146/annurev-ecolsys-110218-024717

Rhen, T., Willingham, E., Sakata, J. T., & Crews, D. (1999). Incubation Temperature Influences Sex-Steroid Levels in Juvenile Red-Eared Slider Turtles, *Trachemys scripta*, a Species with Temperature-Dependent Sex Determination. *Biology of Reproduction*, *61*(5), 1275–1280. https://doi.org/10.1095/biolreprod61.5.1275

Riley, J. L., Freedberg, S., & Litzgus, J. D. (2014). Incubation temperature in the wild influences hatchling phenotype of two freshwater turtle species. *Evolutionary Ecology Research*, *16*(5), 397–416.

Riley, J. L., Hudson, S., Frenette-Ling, C., & Davy, C. M. (2020). All together now! Hatching synchrony in freshwater turtles. *Behavioral Ecology and Sociobiology*, *74*(5), 1–15.

Riley, J. L., & Litzgus, J. D. (2014). Cues used by predators to detect freshwater turtle nests may persist late into incubation. *The Canadian Field-Naturalist*, *128*(2), Article 2. https://doi.org/10.22621/cfn.v128i2.1583

Riley, J. L., Tattersall, G. J., & Litzgus, J. D. (2014). Potential sources of intra-population variation in painted turtle (*Chrysemys picta*) hatchling overwintering strategy. *Journal of Experimental Biology*, jeb.111120. https://doi.org/10.1242/jeb.111120

Ritzel, K., & Gallo, T. (2020). Behavior Change in Urban Mammals: A Systematic Review. *Frontiers in Ecology and Evolution*, *8*. https://www.frontiersin.org/articles/10.3389/fevo.2020.576665

Roberts, H. P., Willey, L. L., Jones, M. T., Akre, T. S. B., King, D. I., Kleopfer, J., Brown, D. J., Buchanan, S. W., Chandler, H. C., deMaynadier, P., Winters, M., Erb, L., Gipe, K. D., Johnson, G., Lauer, K., Liebgold, E. B., Mays, J. D., Meck, J. R., Megyesy, J., … Zarate, B. (2023). Is the future female for turtles? Climate change and wetland configuration predict sex ratios of a freshwater species. *Global Change Biology*, *29*(10), 2643–2654. https://doi.org/10.1111/gcb.16625

Rodrigues, Y. K., & Beldade, P. (2020). Thermal plasticity in insects’ response to climate change and to multifactorial environments. *Frontiers in Ecology and Evolution*, *8*, 271.

Roll, U., Feldman, A., Novosolov, M., Allison, A., Bauer, A. M., Bernard, R., Böhm, M., Castro-Herrera, F., Chirio, L., Collen, B., Colli, G. R., Dabool, L., Das, I., Doan, T. M., Grismer, L. L., Hoogmoed, M., Itescu, Y., Kraus, F., LeBreton, M., … Meiri, S. (2017). The global distribution of tetrapods reveals a need for targeted reptile conservation. *Nature Ecology & Evolution*, *1*(11), 1677–1682. https://doi.org/10.1038/s41559-017-0332-2

Roosenburg, W. M. (1996). Maternal Condition and Nest Site Choice: An Alternative for the Maintenance of Environmental Sex Determination?1. *American Zoologist*, *36*(2), 157–168. https://doi.org/10.1093/icb/36.2.157

Rose, F. L. (2011). Annual Frequency of Clutches of *Pseudemys texana* and *Trachemys scripta* at the Headwaters of the San Marcos River in Texas. *The Southwestern Naturalist*, *56*(1), 61–65. https://doi.org/10.1894/PAS-24.1

Rose, F. L., & Manning, R. W. (1996). Notes on the biology of the slider, *Trachemys scripta elegans* (Reptilia: Emydidae), inhabiting man-made cattle ponds in West Texas. *Texas Journal of Science*, *48*(3), 191–206.

Rosser, A. M., & Mainka, S. A. (2002). Overexploitation and Species Extinctions. *Conservation Biology*, *16*(3), 584–586.

Ruell, E. W., Riley, S. P. D., Douglas, M. R., Antolin, M. F., Pollinger, J. R., Tracey, J. A., Lyren, L. M., Boydston, E. E., Fisher, R. N., & Crooks, K. R. (2012). Urban Habitat Fragmentation and Genetic Population Structure of Bobcats in Coastal

Southern California. *The American Midland Naturalist*, *168*(2), 265–280. https://doi.org/10.1674/0003-0031-168.2.265

Ruhr, I. M., Rose, K. A., Sellers, W. I., Crossley, D. A., & Codd, J. R. (2021). Turning turtle: Scaling relationships and self-righting ability in *Chelydra serpentina*. *Proceedings of the Royal Society B*, *288*(1946), 20210213.

Schubauer, J. P., & Parmenter, R. R. (1981). Winter feeding by aquatic turtles in a southeastern reservoir. *Journal of Herpetology*, *15*(4), 444–447.

Schwanz, L. E., Spencer, R.-J., Bowden, R. M., & Janzen, F. J. (2010). Climate and predation dominate juvenile and adult recruitment in a turtle with temperature-dependent sex determination. *Ecology*, *91*(10), 3016–3026. https://doi.org/10.1890/09-1149.1

Schwarz, C. J., & Arnason, A. N. (1996). A General Methodology for the Analysis of Capture-Recapture Experiments in Open Populations. *Biometrics*, *52*(3), 860–873. https://doi.org/10.2307/2533048

Seber, G. A. F. (1965). A Note on the Multiple-Recapture Census. *Biometrika*, *52*(1/2), 249–259. https://doi.org/10.2307/2333827

Selman, W., Qualls, C., & Owen, J. C. (2013). Effects of human disturbance on the behavior and physiology of an imperiled freshwater turtle. *The Journal of Wildlife Management*, *77*(5), 877–885. https://doi.org/10.1002/jwmg.538

Shine, R., Elphick, M. J., & Harlow, P. S. (1997). The Influence of Natural Incubation Environments on the Phenotypic Traits of Hatchling Lizards. *Ecology*, *78*(8), 2559–2568. https://doi.org/10.1890/0012-9658(1997)078%5B2559:TIONIE%5D2.0.CO;2

Shine, R., & Olsson, M. (2003). When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology*, *16*(5), 823–832. https://doi.org/10.1046/j.1420-9101.2003.00600.x

Shochat, E., Lerman, S. B., Anderies, J. M., Warren, P. S., Faeth, S. H., & Nilon, C. H. (2010). Invasion, Competition, and Biodiversity Loss in Urban Ecosystems. *BioScience*, *60*(3), 199–208. (edsjsr.10.1525.bio.2010.60.3.6). https://doi.org/10.1525/bio.2010.60.3.6

Shochat, E., Stefanov, W. L., Whitehouse, M. E. A., & Faeth, S. H. (2004). Urbanization and Spider Diversity: Influences of Human Modification of Habitat Structure and Productivity. *Ecological Applications*, *14*(1), 268–280. https://doi.org/10.1890/02-5341

Sinervo, B. (1990). The Evolution of Maternal Investment in Lizards: An Experimental and Comparative Analysis of Egg Size and Its Effects on Offspring Performance. *Evolution*, *44*(2), 279–294. https://doi.org/10.2307/2409407

Siviter, H., Charles Deeming, D., Rosenberger, J., Burman, O. H., Moszuti, S. A., & Wilkinson, A. (2017). The impact of egg incubation temperature on the personality of oviparous reptiles. *Animal Cognition*, *20*(1), 109–116.

Smith, G. R., Rettig, J. E., & Iverson, J. B. (2017). Is righting response lateralized in two species of freshwater turtles? *Behaviour*, *154*(9–10), 1069–1079.

Soulsbury, C. D., & White, P. C. L. (2015). Human–wildlife interactions in urban areas: A review of conflicts, benefits and opportunities. *Wildlife Research*, *42*(7), 541. https://doi.org/10.1071/WR14229

Spencer, R.-J. (2002). Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles. *Ecology*, *83*(8), 2136–2144. https://doi.org/10.1890/0012-9658(2002)083%5B2136:ETNSSF%5D2.0.CO;2

Spencer, R.-J., & Thompson, M. B. (2003). The significance of predation in nest site selection of turtles: An experimental consideration of macro- and microhabitat preferences. *Oikos*, *102*(3), 592–600. https://doi.org/10.1034/j.1600-0706.2003.12436.x

Steen, D. A., Aresco, M. J., Beilke, S. G., Compton, B. W., Condon, E. P., Kenneth Dodd Jr., C., Forrester, H., Gibbons, J. W., Greene, J. L., Johnson, G., Langen, T. A., Oldham, M. J., Oxier, D. N., Saumure, R. A., Schueler, F. W., Sleeman, J. M., Smith, L. L., Tucker, J. K., & Gibbs, J. P. (2006). Relative vulnerability of female turtles to road mortality. *Animal Conservation*, *9*(3), 269–273. https://doi.org/10.1111/j.1469-1795.2006.00032.x

Steen, D. A., & Gibbs, J. P. (2004). Effects of Roads on the Structure of Freshwater Turtle Populations. *Conservation Biology*, *18*(4), 1143–1148. https://doi.org/10.1111/j.1523-1739.2004.00240.x

Stemle, L. R., & Searcy, C. A. (2024). Habitat characteristics favoring native freshwater turtles in the highly invaded urban turtle community of Miami-Dade County. *Biological Invasions*, *26*(4), 1181–1194. https://doi.org/10.1007/s10530-023-03236-8

Steyermark, A. C., & Spotila, J. R. (2001). Body temperature and maternal identity affect snapping turtle (*Chelydra serpentina*) righting response. *Copeia*, *2001*(4), 1050–1057.

Stoner, D. C., Wolfe, M. L., & Choate, D. M. (2006). Cougar Exploitation Levels in Utah: Implications for Demographic Structure, Population Recovery, and Metapopulation Dynamics. *The Journal of Wildlife Management*, *70*(6), 1588–1600.

Strickland, J., Colbert, P., & Janzen, F. J. (2010). Experimental Analysis of Effects of Markers and Habitat Structure on Predation of Turtle Nests. *Journal of Herpetology*, *44*(3), 467–470. https://doi.org/10.1670/08-323.1

Sumasgutner, P., Nemeth, E., Tebb, G., Krenn, H. W., & Gamauf, A. (2014). Hard times in the city – attractive nest sites but insufficient food supply lead to low reproduction rates in a bird of prey. *Frontiers in Zoology*, *11*(1), 48. https://doi.org/10.1186/1742-9994-11-48

Sun, B.-J., Li, T., Gao, J., Ma, L., & Du, W.-G. (2015). High incubation temperatures enhance mitochondrial energy metabolism in reptile embryos. *Scientific Reports*, *5*(1), 8861. https://doi.org/10.1038/srep08861

Sun, B.-J., Ma, L., Wang, Y., Mi, C., Buckley, L. B., Levy, O., Lu, H., & Du, W.-G. (2021). Latitudinal embryonic thermal tolerance and plasticity shape the vulnerability of oviparous species to climate change. *Ecological Monographs*, *91*(3), e01468.

Szulkin, M., Munshi-South, J., & Charmantier, A. (2020). *Urban Evolutionary Biology*. Oxford University Press.

Talloen, W., Dyck, H. V., & Lens, L. (2004). The cost of melanization: Butterfly wing coloration under environmental stress. *Evolution*, *58*(2), 360–366. https://doi.org/10.1111/j.0014-3820.2004.tb01651.x

Temple, S. A. (1987). Predation on Turtle Nests Increases near Ecological Edges. *Copeia*, *1987*(1), 250–252. https://doi.org/10.2307/1446069

Thorington, K. K., & Bowman, R. (2003). Predation rate on artificial nests increases with human housing density in suburban habitats. *Ecography*, *26*(2), 188–196. https://doi.org/10.1034/j.1600-0587.2003.03351.x

Tiatragul, S., Hall, J. M., & Warner, D. A. (2020). Nestled in the city heat: Urban nesting behavior enhances embryo development of an invasive lizard. *Journal of Urban Ecology*, *6*(1), juaa001. https://doi.org/10.1093/jue/juaa001

Treidel, L. A., Carter, A. W., & Bowden, R. M. (2016). Temperature experienced during incubation affects antioxidant capacity but not oxidative damage in hatchling red-eared slider turtles (*Trachemys scripta elegans*). *Journal of Experimental Biology*, *219*(4), 561–570. https://doi.org/10.1242/jeb.128843

Tucker, J. K. (2001). Clutch Frequency in the Red-Eared Slider (*Trachemys scripta elegans*). *Journal of Herpetology*, *35*(4), 664–668. https://doi.org/10.2307/1565907

Tucker, J. K., Janzen, F. J., & Paukstis, G. L. (1998). Variation in Carapace Morphology and Reproduction in the Red-Eared Slider *Trachemys scripta elegans*. *Journal of Herpetology*, *32*(2), 294–298. https://doi.org/10.2307/1565315

Tucker, J. K., & Paukstis, G. L. (2000). Hatching Success of Turtle Eggs Exposed to Dry Incubation Environment. *Journal of Herpetology*, *34*(4), 529–534. https://doi.org/10.2307/1565267

Turtle Conservation Coalition, Rhodin, A. G. J., Walde, A. D., Horne, B. D., van Dijk, P. P., Blanck, T., & Hudson, R. (2011). *Turtles in Trouble: The World's 25+ Most*

Endangered Tortoises and Freshwater Turtles—2011. Lunenburg, MA: IUCN/SSC Tortoise and Freshwater Turtle Specialist Group, Turtle Conservation Fund, Turtle Survival Alliance, Turtle Conservancy, Chelonian Research Foundation, Conservation International, Wildlife Conservation Society, and San Diego Zoo Global.

Van Casteren, A., Sellers, W. I., Crossley, D. A., Costello, L. M., & Codd, J. R. (2024). Shell shape does not accurately predict self-righting ability in hatchling freshwater turtles. *Scientific Reports*, *14*(1), 4919. https://doi.org/10.1038/s41598-024-54191-w

Vanek, J. P., & Glowacki, G. A. (2019). Assessing the Impacts of Urbanization on Sex Ratios of Painted Turtles (*Chrysemys picta*). *Diversity*, *11*(5), 72. https://doi.org/10.3390/d11050072

Vega Rabelo, S., Haight, J. D., & Bateman, H. L. (2025). How urban impervious surface shapes bird foraging behavior in an arid city. *PeerJ*, *13*, e19980. https://doi.org/10.7717/peerj.19980

Vetter, S. G., Puskas, Z., Bieber, C., & Ruf, T. (2020). How climate change and wildlife management affect population structure in wild boars. *Scientific Reports*, *10*(1), 7298. https://doi.org/10.1038/s41598-020-64216-9

Vianna, V. R., Costa, G. C., de Alencar, P. R., & Dias, R. I. (2023). Road mortality in the blue-black grassquit (*Volatinia jacarina*) is seasonally driven and sex-biased. *Austral Ecology*, *48*(6), 1154–1167. https://doi.org/10.1111/aec.13325

Vincze, E., Seress, G., Lagisz, M., Nakagawa, S., Dingemanse, N. J., & Sprau, P. (2017). Does Urbanization Affect Predation of Bird Nests? A Meta-Analysis.

Frontiers in Ecology and Evolution, *5*. https://www.frontiersin.org/articles/10.3389/fevo.2017.00029

Voigt, C. C., Phelps, K. L., Aguirre, L. F., Schoeman, M. C., Vanitharani, J., & Zubaid, A. (2016). Bats and buildings: The conservation of synanthropic bats. *Bats in the Anthropocene: Conservation of Bats in a Changing World*, 427–462.

Warner, D. A., & Andrews, R. M. (2002). Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*. *Herpetologica*, *58*(4), 399–407. https://doi.org/10.1655/0018-0831(2002)058%5B0399:NSIRTT%5D2.0.CO;2

Warner, D. A., Mitchell, T. S., & Janzen, F. J. (2024). Increased nest temperature during winter does not affect residual yolk metabolism of hatchling painted turtles (*Chrysemys picta*). *Integrative Zoology*, *00*, 1–11. https://doi.org/10.1111/1749-4877.12890

Warner, D. A., Moody, M. A., Telemeco, R. S., & Kolbe, J. J. (2012). Egg environments have large effects on embryonic development, but have minimal consequences for hatchling phenotypes in an invasive lizard. *Biological Journal of the Linnean Society*, *105*(1), 25–41.

Warner, D. A., & Shine, R. (2008). Maternal nest-site choice in a lizard with temperature-dependent sex determination. *Animal Behaviour*, *75*(3), 861–870. https://doi.org/10.1016/j.anbehav.2007.07.007

Weeks, B. C., Klemz, M., Wada, H., Darling, R., Dias, T., O'Brien, B. K., Probst, C. M., Zhang, M., & Zimova, M. (2022). Temperature, size and developmental plasticity in birds. *Biology Letters*, *18*(12), 20220357.

Weisrock, D. W., & Janzen, F. J. (1999). Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Functional Ecology*, *13*(1), 94–101.

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press.

While, G. M., Noble, D. W. A., Uller, T., Warner, D. A., Riley, J. L., Du, W.-G., & Schwanz, L. E. (2018). Patterns of developmental plasticity in response to incubation temperature in reptiles. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 162–176. https://doi.org/10.1002/jez.2181

White, G. C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations of marked animals. *Bird Study*, *46*(sup1), S120–S139. https://doi.org/10.1080/00063659909477239

Wibbels, T., Cowan, J., & LeBoeuf, R. (1998). Temperature-dependent sex determination in the red-eared slider turtle, *Trachemys scripta*. *Journal of Experimental Zoology*, *281*(5), 409–416. https://doi.org/10.1002/(SICI)1097-010X(19980801)281:5%3C409::AID-JEZ6%3E3.0.CO;2-S

Wilbur, H. M. (1975). The Evolutionary and Mathematical Demography of the Turtle *Chrysemys picta*. *Ecology*, *56*(1), 64–77. https://doi.org/10.2307/1935300

Wilcove, D. S. (1985). Nest Predation in Forest Tracts and the Decline of Migratory Songbirds. *Ecology*, *66*(4), 1211–1214. https://doi.org/10.2307/1939174

Wilhoft, D. C., Del Baglivo, M. G., & Del Baglivo, M. D. (1979). Observations on Mammalian Predation of Snapping Turtle Nests (Reptilia, Testudines, Chelydridae). *Journal of Herpetology*, *13*(4), 435–438. https://doi.org/10.2307/1563478

Wilson, A. D., & Godin, J.-G. J. (2010). Boldness and intermittent locomotion in the bluegill sunfish, *Lepomis macrochirus*. *Behavioral Ecology*, *21*(1), 57–62.

Wilson, D. S. (1998). Nest-Site Selection: Microhabitat Variation and Its Effects on the Survival of Turtle Embryos. *Ecology*, *79*(6), 1884–1892. https://doi.org/10.1890/0012-9658(1998)079%5B1884:NSSMVA%5D2.0.CO;2

Witherington, B. E. (1992). Behavioral Responses of Nesting Sea Turtles to Artificial Lighting. *Herpetologica*, *48*(1), 31–39.

Wolak, M. E. (2012). nadiv: An R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods in Ecology and Evolution*, *3*(5), 792–796. https://doi.org/10.1111/j.2041-210X.2012.00213.x

Xu, Y., Cao, Z., & Wang, B. (2020). Effect of urbanization intensity on nest-site selection by Eurasian Magpies (*Pica pica*). *Urban Ecosystems*, *23*(5), 1099–1105. https://doi.org/10.1007/s11252-020-00996-2

Yntema, C. L. (1979). Temperature levels and periods of sex determination during incubation of eggs of *Chelydra serpentina*. *Journal of Morphology*, *159*(1), 17–27.

Appendix 1: Supplementary figures and tables for Chapter 2

Table 2.1S. Means and standard errors of morphological measurements taken post-hatching, from hatchlings whose eggs were incubated at each of five constant incubation temperatures.

	24 °C	**26 °C**	**28 °C**	**30 °C**	**32 °C**
Carapace length	31.27 ± 0.47	31.60 ± 0.39	31.53 ± 0.38	31.60 ± 0.45	31.14 ± 0.64
Carapace width	29.21 ± 0.54	30.27 ± 0.53	30.18 ± 0.51	30.04 ± 0.55	29.29 ± 0.56
Plastron length	29.44 ± 0.47	30.05 ± 0.40	30.31 ± 0.42	30.07 ± 0.40	30.02 ± 0.56
Plastron width	23.01 ± 0.35	23.46 ± 0.36	23.30 ± 0.46	23.42 ± 0.36	22.89 ± 0.48
Body depth	17.40 ± 0.24	17.28 ± 0.16	17.16 ± 0.20	17.34 ± 0.23	17.55 ± 0.25
Notch-vent	2.80 ± 0.16	2.79 ± 0.12	2.62 ± 0.09	2.82 ± 0.15	3.09 ± 0.25
Vent-tip	10.67± 0.23	10.83 ± 0.22	10.37 ± 0.20	10.10 ± 0.26	9.50 ± 0.20
Mass	8.96 ± 0.33	8.96 ± 0.28	8.65 ± 0.28	9.00 ± 0.30	8.68 ± 0.41

Table 2.2S. Sample sizes per treatment of individuals included in righting response trial analyses.

Treatment		**Number of individuals**
Incubation temperature	Overwinter environment	
24 °C	Aquatic	12
24 °C	Terrestrial	7
26 °C	Aquatic	12

Treatment		Number of individuals
26 °C	Terrestrial	8
28 °C	Aquatic	12
28 °C	Terrestrial	7
30 °C	Aquatic	12
30 °C	Terrestrial	6
32 °C	Aquatic	6
32 °C	Terrestrial	8

Table 2.3S. Full models. Effect of incubation temperature, overwintering environment, and other variables on total righting time, active righting time, and latency to right.

Independent variable	Effect on total righting time	Effect on active righting time	Effect on latency to right
Incubation temperature	$\beta = 5.25e^{-2}$ $(2.90e^{-2})$ p = 0.077	$\beta = 1.17e^{\mathbf{-2}}$ $(5.51e^{-2})$ p = 0.832	$\beta = 3.57e^{-2}$ $(3.43e^{-2})$ p = 0.304
Overwinter environment	$\beta = -2.24e^{-1}(1.81e\text{-}^{2})$ p = 0.223	$\beta = 9.46e^{-2}$ $(3.22e^{-1})$ p = 0.770	$\beta = -4.78e^{-1}$ $(2.03e^{-1})$ p = **0.022**
Inc. temp. * ovw. environ.	$\beta = -6.35e^{-2}$ $(2.98e^{-2})$ p = **0.037**	$\beta = -8.39e^{-3}$ $(5.31e^{-2})$ p = 0.875	$\beta = -5.72e^{-2}$ $(3.26e^{-2})$ p = 0.084
Age at trials	$\beta = -2.10e^{-3}$ $(3.93e^{-3})$ p = 0.601	$\beta = -7.51e^{-3}$ $(7.94e^{-3})$ p = 0.357	$\beta = 4.26e^{-4}$ $(4.65e^{-3})$ p = 0.928
Days in winter housing	$\beta = -1.03e^{-4}$ $(3.30e^{-3})$ p = 0.975	$\beta = 1.28e^{-4}$ $(5.83e^{-3})$ p = 0.983	$\beta = 3.57e^{-4}$ $(3.76e^{-3})$ p = 0.925
PC1	$\beta = 6.64e^{-3}$ $(1.90e^{-2})$ p = 0.728	$\beta = 5.49e^{-3}$ $(3.39e^{-2})$ p = 0.872	$\beta = -1.07e^{-2}$ $(2.28e^{-2})$ p = 0.640

Independent variable	**Effect on total righting time**	**Effect on active righting time**	**Effect on latency to right**
PC2	$\beta = -7.64e^{-2}$ ($5.27e^{-2}$) $p = 0.151$	$\beta = -8.64e^{-2}$ ($9.43e^{-2}$) $p = 0.363$	$\beta = -1.17e^{-1}$ ($5.88e^{-2}$) $p =$ **0.050**
DMIL	$\beta = -4.14e^{\mathbf{-2}}$ ($5.66e^{-2}$) $p = 0.473$	$\beta = -5.69e^{-2}$ ($1.12e^{-1}$) $p = 0.616$	$\beta = 2.35e^{-2}$ ($6.96e^{-2}$) $p = 0.739$
Year	$\beta = -1.17e^{-1}$ ($1.74e^{-1}$) $p = 0.514$	$\beta = -3.89e^{-1}$ ($3.60e^{-1}$) $p = 0.302$	$\beta = 1.50e^{-2}$ ($2.14e^{-1}$) $p = 0.945$

Note: For the overwinter environment variable, ‘Aquatic’ is the reference. For the year variable, ‘2019’ is the reference. Standard errors are noted in parentheses.

Table 2.4S. Full models. Effect of incubation temperature, overwintering environment, spring hatchling measurements, and other variables on total righting time and latency to right.

Independent variable	**Effect on total righting time**	**Effect on active righting time**	**Effect on latency to right**
Incubation temperature	$\beta = 0.061$ (0.033) $p = 0.070$	$\beta = 0.012$ (0.059) $p = 0.846$	$\beta = 0.009$ (0.037) $p = 0.811$
Overwinter environment	$\beta = -0.297$ (0.184) $p = 0.114$	$\beta = -0.027$ (0.334) $p = 0.935$	$\beta = -0.467$ (0.206) $p =$ **0.027**
Inc. temp. * ovw. environ.	$\beta = -0.067$ (0.031) $p =$ **0.035**	$\beta = 0.011$ (0.056) $p = 0.841$	$\beta = -0.051$ (0.034) $p = 0.141$
Age at trials	$\beta = -0.003$ (0.005) $p = 0.539$	$\beta = -0.009$ (0.008) $p = 0.274$	$\beta = 0.004$ (0.005) $p = 0.437$
Days in winter housing	$\beta = -0.001$ (0.003) $p = 0.816$	$\beta = 0.001$ (0.006) $p = 0.860$	$\beta = -0.001$ (0.004) $p = 0.824$
DMIL	$\beta = -0.036$ (0.062)	$\beta = -0.032$ (0.114)	$\beta = 0.010$ (0.070)

Independent variable	**Effect on total righting time**	**Effect on active righting time**	**Effect on latency to right**
	p = 0.565	p = 0.781	p = 0.886
Year	β = -0.125 (0.203) p = 0.552	β = -0.527 (0.372) p = 0.183	β = 0.099 (0.219) p = 0.659
Carapace length (mm)	β = -0.049 (0.066) p = 0.455	β = 0.201 (0.119) p = 0.097	β = -0.166 (0.074) p = **0.028**
Carapace width (mm)	β = 0.120 (0.064) p = 0.066	β = -0.101 (0.116) p = 0.390	β = 0.084 (0.067) p = 0.213
Plastron length (mm)	β = -0.041 (0.050) p = 0.411	β = -0.212 (0.090) p = **0.021**	β = 0.039 (0.055) p = 0.488
Plastron width (mm)	β = -0.046 (0.059) p = 0.431	β = 0.152 (0.106) p = 0.157	β = -0.074 (0.066) p = 0.270
Body depth (mm)	β = -0.045 (0.090) p = 0.623	β = -0.069 (0.164) p = 0.673	β = 0.038 (0.098) p = 0.700
Notch-vent (mm)	β = -0.163 (0.074) p = **0.033**	β = -0.189 (0.135) p = 0.166	β = -0.139 (0.084) p = 0.105
Vent-tip (mm)	β = 0.001 (0.052) p = 0.990	β = 0.017 (0.094) p = 0.857	β = -0.054 (0.061) p = 0.380
Mass (g)	β = 0.039 (0.048) p = 0.414	β = -0.007 (0.086) p = 0.939	β = 0.109 (0.054) p = **0.046**

Note: For the overwinter environment variable, ‘Aquatic’ is the reference. For the year variable, ‘2019’ is the reference. Standard errors are noted in parentheses.

Table 2.5S. Final models: effect of incubation temperature, overwintering environment, spring hatchling measurements, and other variables on total righting time and latency to right (after backwards stepwise regression).

Independent variable	**Effect on total righting time**	**Effect on latency to right**
Incubation temperature	β = 0.058 (0.019) p = **0.003**	β = 0.025 (0.024) p = 0.301
Overwinter environment	β = -0.179 (0.079) p = **0.027**	β = -0.508 (0.103) p = **4.950e^{-6}**
Inc. temp * ovw. environ.	β = -0.061 (0.028) p = **0.032**	β = -0.046 (0.030) p = 0.130
Carapace length (mm)	-	β = -0.098 (0.041) p = **0.020**
Notch-vent (mm)	-	β = -0.178 (0.073) p = **0.017**
Mass (g)	-	β = 0.107 (0.042) p = **0.013**

Note: For the overwinter environment variable, 'Aquatic' is the reference. The Active righting response model had no remaining fixed effects with statistical significance following the removal of nonsignificant fixed effects. Standard errors are noted in parentheses.

Table 2.6S. Log base 10 transformed models included in DHARMa model inspection process: effect of incubation temperature and overwintering location on hatchling phenotypes after overwintering (i.e., spring measurements).

Independent variable	**Carapace length (mm)**	**Plastron length (mm)**	**Body mass (mm)**
Incubation temperature	β = 4.34e^{-3} (1.80e^{-3}) p = **0.019**	β = 3.90e^{-3} (1.57e^{-3}) p = **0.015**	β = 1.68e^{-2} (5.67e^{-3}) p = **0.004**
Overwinter environment	β = -2.39e^{-2} (1.09e^{-2}) p = **0.031**	β = -2.07e^{-2} (9.54e^{-3}) p = **0.033**	β = -8.83e^{-2} (3.40e^{-2}) p = **0.011**

Independent variable	**Carapace length (mm)**	**Plastron length (mm)**	**Body mass (mm)**
Inc. temp * ovw. environ.	$\beta = -4.57e^{-3}$ ($2.58e^{-3}$) p = 0.080	$\beta = -4.00e^{-3}$ ($2.27e^{-3}$) p = 0.081	$\beta = -1.64e^{-2}$ ($8.06e^{-3}$) p = **0.045**
Previous measurement	$\beta = 1.70e^{-2}$ ($1.81e^{-3}$) p = $\mathbf{1.27e^{-14}}$	$\beta = 1.57e^{-2}$ ($1.63e^{-3}$) p = $\mathbf{3.69e^{-15}}$	$\beta = 5.94e^{-2}$ ($8.17e^{-3}$) p = $\mathbf{1.66e^{-10}}$
Days in winter housing	$\beta = 6.22e^{-4}$ ($2.26e^{-4}$) p = **0.007**	$\beta = 4.22e^{-4}$ ($2.00e^{-4}$) p = **0.038**	$\beta = 1.82e^{-3}$ ($7.03e^{-4}$) p = **0.012**

Note: Random effects of clutch identity were included in all of these models. Standard errors are noted in parentheses.

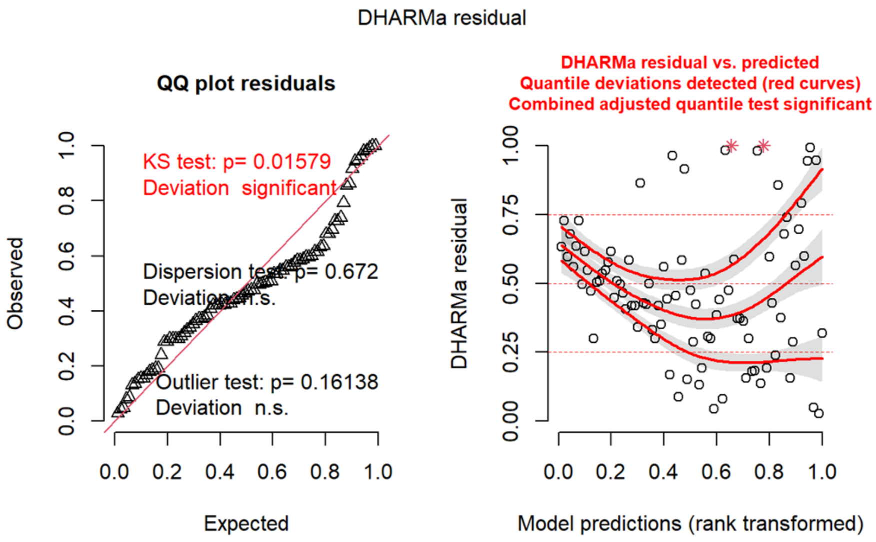

Figure 2.1S. Residuals plots from the 'carapace length' model in Table 3, obtained from the DHARMa package.

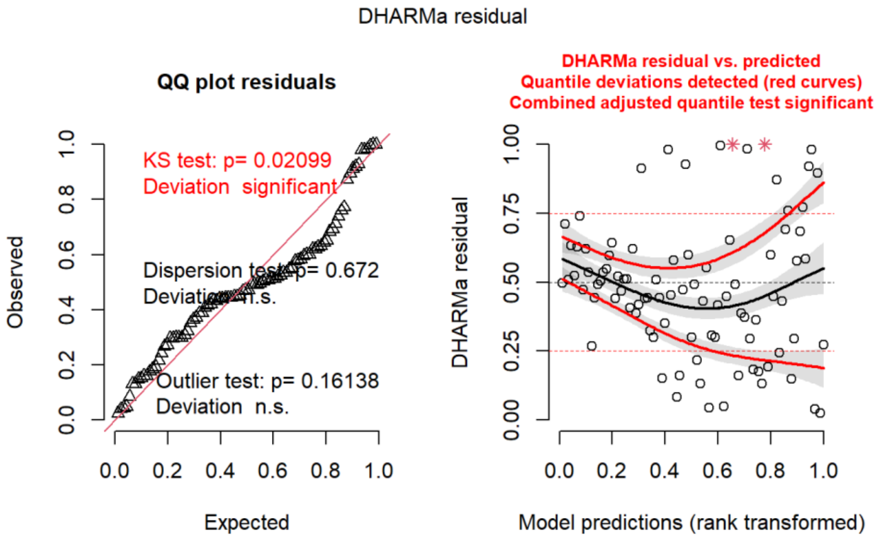

Figure 2.2S. Residuals plots from the 'carapace length' model in Table 3 after a log (base 10) transformation of the response variable, obtained from the DHARMa package.

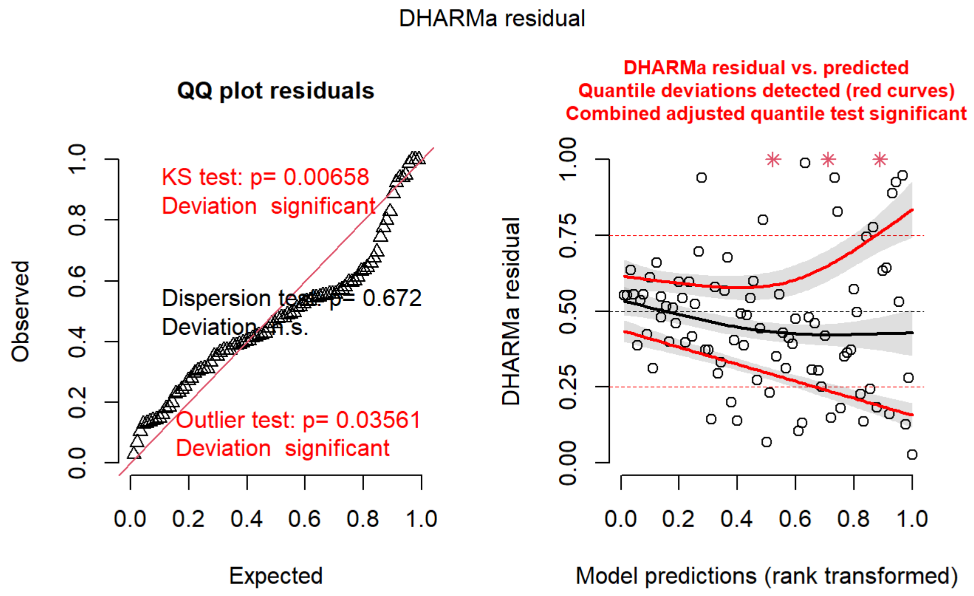

Figure 2.3S. Residuals plots from the ‘plastron length’ model in Table 3, obtained from the DHARMa package.

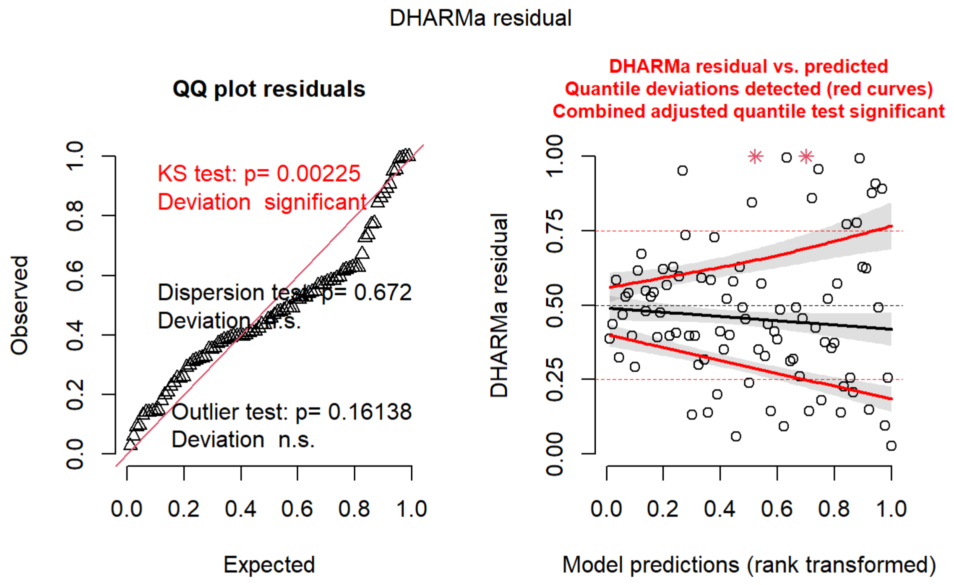

Figure 2.4S. Residuals plots from the ‘plastron length’ model in Table 3 after a log (base 10) transformation of the response variable, obtained from the DHARMa package.

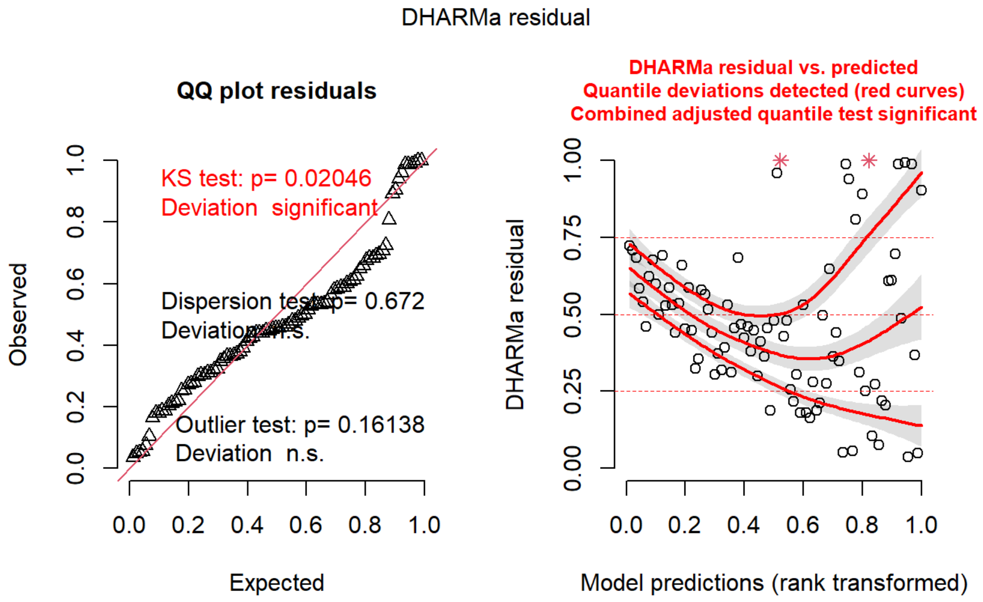

Figure 2.5S. Residuals plots from the 'body mass' model in Table 3, obtained from the DHARMa package.

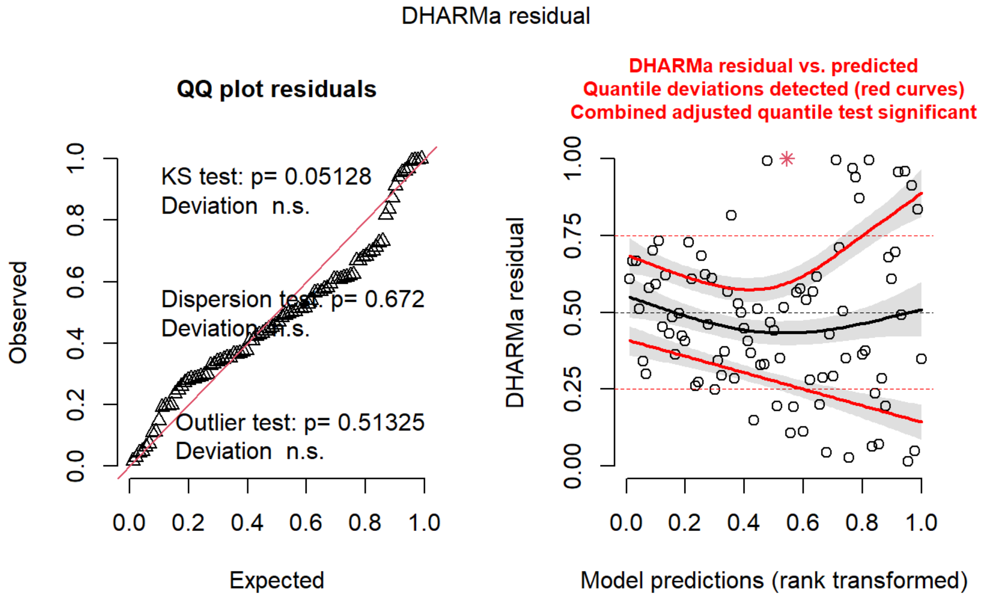

Figure 2.6S. Residuals plots from the 'body mass' model in Table 3 after a log (base 10) transformation of the response variable, obtained from the DHARMa package.

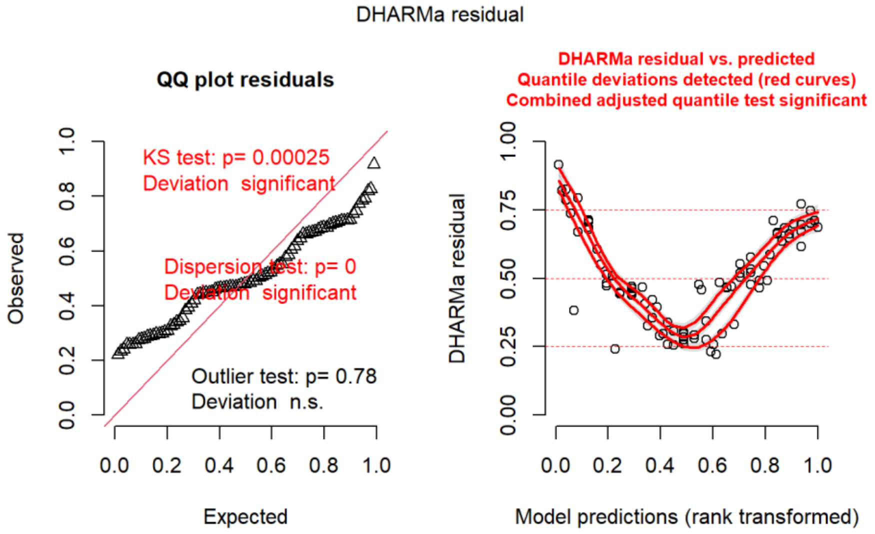

Figure 2.7S. Residuals plots from the ‘incubation duration’ model in Table 2, obtained from the DHARMa package.