

**Causes and consequences of variation in maternal investment strategies**

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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## **DEDICATION**

I dedicate this dissertation to Dan Warner, my MS advisor. This dissertation was more enjoyable because of the academic skills he helped me develop.

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## ABSTRACT

Females across taxa increase fitness by investing resources and care to offspring. However, numerous intrinsic and extrinsic factors influence both female and offspring performance and survival. Such complexity leads to tradeoffs in investment and variety in investment strategies. For iteroparous species, females must balance investment into reproduction with their own survival and future reproduction. In this dissertation, I examine how females balance numerous pressures on investment with an emphasis on how investment strategies vary across life.

In chapter two, I tested how maternal predation risk influences nest-site choice, and how maternal responses to risk affect offspring survival in painted turtles (*Chrysemys picta*). I compared young and old mothers to assess if response to risk varies depending upon maternal age. I predicted that young mothers would invest heavily in themselves (i.e., nest closer to the safety of water) whereas older mothers would invest more into current reproduction (i.e., nest farther from the shore because of lower nest predation risk). Contrary to predictions, neither young nor old females altered how far they nested from water after perceiving elevated risk. Nevertheless, older females nested farther from water than younger females, which is likely driven by lower future reproductive potential (i.e., residual reproductive value, RRV) in older females.

In chapter three, I assessed how offspring phenotype influences investment strategy in common snapping turtles (*Chelydra serpentina*). I released hatchling turtles at varying distances from water and monitored survival during overland dispersal. Survival decreased with dispersal distance. However, bigger hatchlings were less affected by increasing dispersal distance. Moreover, females producing larger and better dispersing offspring oviposited farther from water

than females that produced smaller and poorer dispersing offspring. These findings suggest female investment can be sensitive to offspring phenotype and that such covariation between nest-site choice and offspring dispersal ability can maximize offspring survival and, thus, maternal fitness.

In chapter four, I examined how age and RRV compare in explaining variation in a risky investment behavior (i.e., distance females construct nests from water) in painted turtles. Previous work (in addition to chapter 2) has shown that older females nest farther from water than younger mothers and suggested this effect is driven by RRV. I predicted that RRV would explain more variation in distance to water than age because RRV accounts for any nonlinearity in future reproductive potential across age. Contrary to my prediction, age was a better predictor of nest distance to water than RRV. This finding suggests a stronger correlate of age (e.g., body size) may be more responsible for shaping the distance females nest from water than previously appreciated.

Collectively, this work suggests nest-site choice is a complex behavior that is shaped by numerous factors, many of which interact. Moreover, investment strategies shift across age to maximize lifetime fitness in freshwater turtles.

## CHAPTER 1: AN INTRODUCTION TO THE DISSERTATION

The phenotype of an organism is shaped by its genotype and the environment it experiences during development and throughout life (Gilbert and Epel 2009). Mothers thus have a large effect on offspring phenotype through the transfer of DNA. However, maternal experience and behavior can also greatly affect offspring phenotype by influencing the developmental environment of offspring (i.e., maternal effects; Mousseau and Fox 1998). For example, the photoperiod and temperatures experienced by females of many insects influence the proportion of their offspring that delay development until environmental conditions improve (i.e., diapause; Mousseau and Dingle 1991). In addition, the quality and quantity of food consumed by mothers influence offspring body size, behavior, and performance in a variety of taxa (Massot and Clobert 1995; Moczek 1998; Grindstaff et al. 2005; Warner et al. 2007, 2015; Sullivan et al. 2014). Another important maternal effect is the location that a mother chooses to oviposit eggs or give birth (Bernardo 1996; Refsnider and Janzen 2010). In fact, nest environment is often the greatest determinant of early-life success in oviparous taxa (Bernardo 1996). For example, oviposition sites can influence offspring growth (Grossmueller and Lederhouse 1985; Kouki 1993; Mitchell et al. 2015), predation risk (Rieger et al. 2004; Rearden et al. 2011), and even sex determination (i.e., environmental sex determination; Shine 1999; Janzen 1994a, 1994b; Refsnider and Janzen 2010). Therefore, maternal behaviors are major targets of natural selection because of their profound effects on the fitness of offspring and parents.

Theory predicts that iteroparous organisms should balance investment into current reproduction with their own survival and future reproduction to maximize lifetime fitness (Williams 1966; Gadgil and Bossert 1970; Pianka and Parker 1975; Clutton-Brock 1984; Roff

1992; Stearns 1992). Specifically, organisms should increase investment into reproduction as their future reproductive opportunities decrease (i.e., residual reproductive value, RRV, Williams 1966; Pianka and Parker 1975). In support of this contention, numerous taxa increase aspects of investment as they age (Creighton et al. 2009; Hoffman et al. 2010; Weladji et al. 2010; Krams et al. 2011) including humans (Schlomer and Belsky 2012). Moreover, immune challenge often increases reproductive effort in birds (Bonneaud et al. 2004; Hanssen 2006; Velando et al. 2006; Bowers et al. 2012) and rodents (Weil et al. 2006), presumably because they perceive a decrease in the likelihood of future reproduction. Thus, animals are capable of plastic adjustment of investment to maximize lifetime fitness depending upon RRV. Although theory would predict the magnitude and direction of such plastic investment should vary across age, this prediction is largely untested (Candolin 1998).

Despite a 54-year old prediction that reproductive effort should increase as RRV decreases (Williams 1966), few studies have quantified RRV to test this theory. Age-specific survival and fecundity estimates are required to calculate RRV, which may preclude many researchers from using RRV in models. Instead, ecologists and evolutionary biologists intuitively use age as a proxy for RRV and assume young animals have high RRV that declines with age until death. In reality, RRV often increases during early reproductive life until reproductively prime years and then decreases until death (Pianka and Parker 1975; Vahl 1981; Bayne et al. 1983; Thompson 1984; Begon et al. 1990). Thus, researchers should not simply interpret age-related changes in investment to be driven by RRV. Instead, research that compares the explanatory power of RRV with age may better examine drivers of age-specific reproductive strategies. Moreover, comparing aspects of reproductive effort with RRV instead of age would more directly test Williams' (1966) life-history theory.



Another predicted mechanism to maximize fitness is to tailor investment to the phenotype of a female's offspring. For example, offspring with a higher desiccation-prone morphology or physiology may need to be oviposited in a more humid environment than offspring with anti-desiccation phenotypes. Previous work has tested the specificity of maternal investment by splitting clutches and placing a portion of eggs into maternal nests and another portion into nests of non-related females (Shine et al. 1997; Mitchell et al. 2013). These studies have shown that females choose nests environments that generally increase offspring success, but unrelated nests are as equally beneficial as maternal nests. Regardless, much phenotypic variation exists in offspring traits (e.g., size) among and within populations. When offspring phenotypes differentially benefit by various parental investment strategies, we may expect females to invest in offspring depending upon the specific phenotype of their offspring.

### *Study system*

Turtles are well suited to addressing these foundational concepts in life-history evolution. Most turtles spend the majority of their lives in bodies of water, but venture onto land to construct terrestrial nests. Investment typically ends after nest construction (i.e., no post-oviposition maternal care; but see Iverson 1990; Agha et al. 2013; Ferrara et al. 2013). Embryos incubate in subterranean nests, hatch from eggs, emerge from the nests, and then disperse to water. Survival during these early-life stages is often low because of predation and abiotic stressors (Janzen 1993; Congdon et al. 1999; Tucker 2000; Janzen et al. 2000a; Janzen et al. 2000b; Kolbe and Janzen 2001, 2002; Spencer 2002; Strickland et al. 2010; Mitchell et al. 2013; Mitchell et al. 2017; reviewed in Iverson 1991). Temperate turtles grow yearly rings (i.e., annuli) on the scutes of their shells, which can be visible and countable in many species, especially when

young. Researchers can uniquely and permanently mark hard-shelled turtles by notching the marginal scutes of their carapace allowing us to track the investment history of individuals throughout their lives. In addition, turtles are iteroparous and relatively long lived, which allows researchers to examine reproductive ecology across a wide age range.

Painted turtles (*Chrysemys picta*) are well suited for studies that require observation and identification of mothers because females are observable while searching and constructing nests and finish nesting in a relatively short time period (average nest construction time of 97 min. in Nebraska, Frye et al. 2017). Painted turtles nest mostly during the day, especially during afternoon and evening. Thus, researchers can find females nesting, observe until turtles finish, and then capture them to record identity, phenotype, and reproductive output. Common snapping turtles (*Chelydra serpentina*) on the other hand construct nests mainly at night and over much longer time frames. Common snapping turtles lay clutches of 24–85 eggs in Illinois (St. Juliana et al. 2004; Delaney and Janzen 2019) with a mean of  $52 \pm 3.5$  SD (Kolbe and Janzen 2001), which make them ideally suited for experimental studies that require large quantities of eggs or hatchlings for treatments.

I conducted research at the Thomson Causeway Recreation area along the Mississippi River near Thomson, Illinois. Painted turtles occur at high densities in the backwaters of the River and nest in relatively open-canopy areas with sparse vegetations. Coincidentally this island habitat has been created and maintained by the Army Corps of Engineers as a campground and recreation area. Since 1988, Fredric Janzen and students have studied the nesting ecology of painted turtles in the South Potters loop of the campground. Turtles have been uniquely marked, reproductive output measured, and nest fate monitored (e.g., Refsnider and Janzen 2016). The

mainland to the east consists of sand prairie along the River and is used primarily by snapping turtles to nest.

For my dissertation, I examined the causes and consequences of variation in maternal investment strategies across the reproductive lifespan of freshwater turtles. In chapter one, I exposed nesting painted turtles to simulated predation in the field to examine subsequent nesting behavior and its consequences for nest survival. This experiment tested whether investment in nest-site choice shifts because of risk to mothers and whether such plasticity might vary as females age. In chapter two, I conducted a dispersal experiment in the wild to examine the role of offspring dispersal ability in contributing to the nest-site choice of common snapping turtles. Evidence that parents tailor investment to their offsprings' phenotypes is lacking, despite predictions that such a strategy would be adaptive. In chapter three, I used a 30-year dataset of painted turtle nesting ecology to compare age and RRV in predicting a risky investment behavior, distance of a nesting event from water. This study calls for a reevaluation of how researchers interpret the drivers of variation in age-specific reproduction. Collectively, this work assesses multiple predictions of life-history theory and challenges our interpretations of age-specific reproductive tactics.

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**CHAPTER 2. RISK-SENSITIVE MATERNAL INVESTMENT: AN EVALUATION OF  
PARENT-OFFSPRING CONFLICT OVER NEST-SITE CHOICE IN THE WILD**

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**Abstract**

Parents increase their fitness by investing resources to offspring. However, such investment is costly for parents, leading to tradeoffs, which should shift towards heavier investment to reproduction as females age and future reproductive opportunities decrease. Nests of aquatic turtles laid farther from water have higher survival than those laid closer to shore because nest predators often forage along environmental edges. However, the predation risk of adult females increases farther from water because water is used as refuge from terrestrial predators. Thus, females may balance investment in current offspring vs. maternal survival and future offspring. To test if investment varies depending upon perceived risk, we exposed 30 painted turtles (*Chrysemys picta*) to simulated predation by capturing and handling them shortly

after females chose a nest site. We then released females, which fled to water, and allowed them to return to land and nest undisturbed. We compared the distance to water of nests laid before and after simulated predation. Unexpectedly, females did not vary distance to water in response to simulated predation. Regardless, nest sites chosen after simulated predation were more likely to be depredated than those chosen before simulated predation, suggesting females altered nest-site choice in ways we did not quantify. In addition, although older turtles nested almost twice as far from water as younger turtles, we found no evidence that age influenced maternal response to simulated predation. Our findings suggest perceived risk of mothers to predation influences nest-site choice and subsequently reduces offspring survival in *C. picta*. In addition, we provide a rare assessment of how plastic maternal investment might vary across reproductive life.

### **Introduction**

Parents increase their fitness by investing resources into the production and care of offspring. However, such investment costs energy (Wade and Schneider 1992), reduces immune function (Festa-Bianchet 1989; Durso and French 2018), and elevates risk of predation of parents (Montgomerie and Weatherhead 1988; Magnhagen 1991; Lima 2009). For iteroparous species, parents must balance the benefits of current reproduction with the costs to future reproduction to maximize lifetime fitness. For example, parents may choose to minimally invest in a reproductive bout when environmental conditions are poor so they can allocate more resources to future reproduction when the likelihood of success improves (Reale and Festa-Bianchet 2000). Parent-offspring conflict exists in such situations when the amount of parental investment is not equally beneficial for both parent and offspring fitness (Trivers 1974; Godfray 1995).

Theoretical predictions of parent-offspring conflict have typically been evaluated over post-birth food allocation in birds and mammals (Mock and Forbes 1992; Maestripieri 2002). In these taxa, offspring signal to parents (e.g., by gaping the mouth and/or vocalizing) requesting food that parents must decide to seek and divide among offspring (e.g., Christe et al. 1996). In contrast, how parent-offspring conflict may occur over prenatal maternal investment is understudied (but see Godfray et al. 1991; Eium and Fleming 2000; Ghalambor and Martin 2001; Eggers et al. 2006; Janzen and Warner 2009). Because unborn offspring in oviparous species cannot entice parents to disproportionately invest resources, conflict over prenatal investment should be governed entirely by parents.

Life-history theory predicts that the cost of current reproduction tolerated by parents should scale negatively with future reproductive opportunities (Roff 1992; Stearns 1992). Indeed, long-lived species of birds with low fecundity invest more heavily in their own survival in response to predation risk compared to highly fecund species with shorter lifespans (Ghalambor and Martin 2000, 2001; LaManna and Martin 2016). Similar intraspecific variation in life-history strategies is predicted by the terminal investment hypothesis, which posits that reproductive effort should increase as the likelihood of future reproduction decreases (Williams 1966; Gadgil and Bossert 1970; Pianka and Parker 1975; Clutton-Brock 1984), including within a season (Schneider and Griesser 2015). Indeed, immune challenge can increase reproductive effort in birds (Bonneaud et al. 2004; Hanssen 2006; Velando et al. 2006; Bowers et al. 2012; but see Griesser et al. 2017) and rodents (Weil et al. 2006), presumably because of a diminished likelihood of future reproduction. Alternatively, birds may forgo a reproductive bout (Scheuerlein et al. 2001) or invest less in clutch size/number or nestlings (Harris 1980; Harfenist and Ydenberg 1995; Eggers et al. 2006; Scheuerlein and Gwinner 2006; Thomson et al. 2006;

LaManna and Martin 2016, 2017; Griesser et al. 2017) when environmental conditions are poor, to increase their chances of future reproduction. Thus, animals alter investment in response to mortality risk to maximize lifetime fitness. Furthermore, the strength and direction of those responses (safety vs. reproduction) should vary across age for iteroparous species. Yet, despite extensive theoretical and empirical study of variation in investment across age, it is unknown whether plastic investment changes as individuals senesce and the likelihood of future reproduction decreases (Candolin 1998).

Aquatic turtles are well suited to address these core issues in evolutionary ecology. Maternal investment ends after nest-site choice and oviposition, allowing quantification of investment. Because water is used as a refuge during terrestrial nesting forays (Polich and Barazowski 2016; Delaney et al. 2017) and predation risk increases with distance from refuge across animals (Stankowich and Blumstein 2005), predation risk of adult females should increase with distance to water (Spencer 2002; but see Refsnider et al. 2015). Conversely, embryo survival typically increases with distance to water, because nest predators (e.g., raccoons) often forage along environmental edges (Spencer 2002; Kolbe and Janzen 2002; Strickland et al. 2010). The strength of this effect varies by year at our site, and the benefits of reduced predation risk typically level off after the first 25 to 40 m from water (Kolbe and Janzen 2002). Importantly, if embryos hatch, they must emerge from the nest and disperse to water. Hatchling survival during dispersal decreases the farther offspring must travel to water, but this effect is reduced as hatchling body size increases (Delaney and Janzen 2019). Thus, older mothers, which produce larger offspring (Harms et al. 2005), may have greater flexibility in nesting strategy compared to younger mothers (Delaney and Janzen 2019). Thus, among numerous factors affecting nest-site choice, conflict should exist because maternal survival typically benefits from

nesting closer to water, whereas embryo survival (via probability of nest predation) generally increases farther from water. Furthermore, the long reproductive lifespan of many turtles (e.g., at least 20 years for female painted turtles in Illinois (Warner et al. 2016) and up to about 50 years in Michigan (Congdon et al. 2003)) enables assessment of investment tradeoffs across a wide age range.

We exposed nesting painted turtles (*Chrysemys picta*) to simulated predation events to examine subsequent nesting behaviour and its consequences for offspring survival (as measured primarily by nest predation). Based on the life-history and system-specific theory outlined above, we tested several predictions in this system: (1) Mothers would nest closer to water after simulated predation because predation risk is lower when refuge is close; (2) Mothers would nest in locations with higher nest predation and lower hatching success following simulated predation because females would choose nest sites that prioritize their survival over current offspring survival; (3) Responses to simulated predation would be attenuated in older females because older females would invest more into current reproduction and less into their own survival compared to younger females (i.e., terminal investment hypothesis); and (4) Embryos oviposited by females that experienced simulated predation would have lower hatching success than control eggs because delaying oviposition may have negative embryonic consequences (Ewert 1985; Reynard and Savory 1999; but see Warner and Andrews 2003).

## Methods

We monitored *C. picta* behaviour at the Thomson Causeway Recreation Area in Illinois along the Mississippi River during May–June 2017. The site consists of a grassy area, a rocky shoreline, and a forest up to 75 m from the water. Aside from the rocky shoreline (where turtles

do not nest), the field site is similar in elevation and slope (relatively flat) with the full spectrum of canopy cover options distributed across the area (including from the shoreline to the forest) (similar area as described in Janzen and Morjan 2001; Kolbe and Janzen 2002). Nests take up a small area (<15 cm diameter each) compared to the available nesting range (4 ha), and although potential nesting locations are likely not limited, optimal nest sites may be.

We located nesting females by visually surveying known nesting areas once per hour from 0700–2000 hrs. Care was taken to maximize the distance ( $\geq 15$  m) between observers and females searching for nest sites to minimize observers being perceived as threats prior to the experimental application (Refsnider et al. 2015). We considered a female to have chosen a nest site once she excavated a cavity at least 3 cm deep (nest-site 1). Females that have invested this much energy into nest construction rarely choose a new location for nesting unless they hit impassable substrate or are disturbed by humans or other animals (Delaney et al. 2017).

We then captured the female (before oviposition) and counted growth annuli on the pectoral scutes of the plastron to estimate age. *Chrysemys picta* at this field site have visible growth annuli up to about 8 years of age, after which the early growth annuli fade and become uncountable. Thus, we binned female age into two categories based on the number and fading of growth annuli. ‘Young’ females had 7 to 8 growth annuli and were likely in their first or second year of reproduction, and ‘old’ females had annuli that were too faded to estimate and had likely been reproductively mature for more than two years (sensu Bowden et al. 2004; Harms et al. 2005). Females in the ‘old’ category encompassed a much greater age range than ‘young’ females and may therefore exhibit greater variation in investment (females at this site have been documented to age 29). We ran exploratory analyses with body size, which correlates with age (Hoekstra et al. 2018), in place of age. However, because females reach asymptotic growth at 6-8

years of age (Hoekstra et al. 2018), this analysis does not fully account for the continuous distribution of age either. Nevertheless, we found similar effects using body size in place of the age bin classification and present only the analyses with age bin for simplicity. We painted an identification number on the carapace to allow remote identification (Rust-Oleum oil-based paint; Moldowan et al. 2015). This level of handling for capturing, annuli counting, and marking significantly elevates circulating levels of the stress hormone corticosterone in *C. picta* (Polich 2016) and is similar to predation attempts during which the turtle may be overturned, bitten, and scratched (Bateman et al. 2014). After processing (about 5 minutes in total), we released the turtle at nest-site 1 and allowed her to flee to water.

When paint-marked females (i.e., previously disturbed during nesting) returned to nest, we allowed them to oviposit undisturbed (nest-site 2). We measured the distances between nest sites and the nearest water and forest edge with a laser rangefinder (to nearest m; Nikon Aculon). We also measured the distance between nest-sites 1 and 2 with the rangefinder. We then excavated the eggs, split clutches into two groups, and placed them (and temperature loggers; see below) in artificial nests adjacent to nest-sites 1 and 2 (sensu Mitchell et al. 2013; Bodensteiner et al. 2015). We used spoons and garden trowels to construct artificial nests 10 cm deep, which is near the average nest depth at this site (Refsnider et al. 2013). To eliminate effects of incubating eggs with only half a clutch, we added replacement eggs to each artificial nest from undisturbed females that contemporaneously nested naturally elsewhere within the study area (Mitchell et al. 2013). We wrapped each half clutch in nylon mesh to identify clutch origin after hatching (sensu Mullins 2002). Because embryos derive all energy from yolk stores while in the nest and activity is presumably restricted to development within the egg, hatching, and nest emergence (i.e., no feeding until turtles reach water; Ernst and Lovich 2009), conflict should not exist between

experimental and replacement eggs within nests as might be expected in birds. Instead, this design enabled examination of any effects of delayed oviposition for experimental females.

We protected each nest by burying hardware cloth about 4 cm below the surface of the ground but above the nest cavity, which maintained the visual appearance of natural nests. We then monitored the ground surface for predation attempts (scored as depredated if disturbed). Raccoons are the main predator of nests at this site (Strickland et al. 2010; Voves et al. 2016). We excavated nests from 15–17 September 2017 to examine egg survival due to the abiotic environment. We considered eggs that did not hatch to have perished because of poor environmental conditions, although some may have been inviable or may have succumbed to congenital defects or pathogens. Thus, we were able to parse out variation in the probability of nest survival due to predation risk, the abiotic environment, and delayed oviposition. We calculated nest survival as the number of live hatchlings divided by the number of eggs originally deposited in the nest. We checked each hatchling for shell abnormalities (e.g., extra or fused scutes), but did not assess sex because of permitting restrictions. After processing, we released hatchlings at the edge of the nearest water.

We conducted these behavioural trials on 11 young and 19 old females. It was not possible to record data blind because our study involved focal animals in the field. This research adhered to the ASAB/ABS guidelines for the use of animals in research.

Thermal conditions experienced by ectotherms during incubation have important consequences for embryonic survival and phenotype (Birchard 2004; Noble et al. 2018). Thus, we were secondarily interested how maternal perceived risk might affect the thermal environment of embryos. Just after oviposition, we took a hemispherical photo above each nest to quantify canopy cover and transmitted solar radiance. Canopy openness and solar



transmittance were quantified using Gap Light Analyzer software (Cary Institute of Ecosystem Studies, Millbrook, New York). Solar transmittance was calculated as the average percent of solar transmittance during incubation. We also placed a data logger (iButton to log hourly temperatures) in the middle of each artificial nest to quantify thermal variation between nest sites. We analyzed thermal data from the day after a nest was constructed until August, with exact dates depending on when the iButton was launched and when memory was filled. However, we analyzed the same dates within nest blocks (nest-site 1 and 2 from the same female) to control for thermal variation due to season. Thus, thermal data were analyzed for an average of 72.5 days ( $\pm 13.5$  SD; range 45–85).

Because the temperatures experienced by embryos of *C. picta* determine into which sex they develop (i.e., temperature-dependent sex determination; Janzen 1994a, 1994b), we also analyzed thermal variation during the developmental period when sex (and organogenesis in general) is sensitive to temperature (temperature-sensitive period; TSP). To determine the TSP, we converted Yntema (1968) developmental stages 15–22 for *Chelydra serpentina*, which Bull and Vogt (1981) found to be the TSP in *C. picta*, to Cordero and Janzen (2014) stages 15–19 for *C. picta*. We estimated that Cordero and Janzen stages 15–19 would have occurred about 14–42 days after oviposition in our study (estimated from Figs. 10 and 11 in Cordero and Janzen 2014). We then calculated the constant temperature equivalent (CTE) during this period, which explains turtle sex ratios better than mean temperature alone by accounting for variation in developmental rate due to fluctuating thermal conditions in natural nests (Georges 1989; Georges et al. 1994; code used from Telemeco et al. 2013).

### *Statistical Analysis*

We conducted all analyses in R (version 3.6.0) using package lme4. Mixed model analyses assessing maternal behaviour and offspring consequences included maternal ID as a random effect. We used simulated predation (nest site chosen before vs. after), maternal age (young vs. old), and their 2-way interaction as independent variables in all general and generalized linear mixed models. Thus, we used the following model framework:

Dependent variable = Simulated predation + Maternal age + Simulated predation\*Maternal age +  
 Maternal ID (random effect)

We examined female response to simulated predation with a series of general linear mixed models using the following dependent variables: distance nests were laid from water, distance nests were laid from a forest edge, distance to the nearest water or forest edge, and the CTE during the TSP. Because other nest thermal variables (average, minimum, and maximum temperature, canopy openness, and solar radiation) were often correlated (Supplementary Table 1), we calculated principal components (PC) and used the first 3 as dependent variables. Principal components explained 51%, 26%, and 19% of the variation, respectively, totaling 96%. All variables loaded in the same direction for PC 1, which represented overall thermal variation (Supplementary Table 2). Principal component 2 most strongly captured an inverse relationship between minimum and maximum nest temperatures, whereas PC 3 captured an inverse relationship between minimum nest temperature and canopy openness. We assessed the type of soil chosen by females with a generalized linear mixed model and a logit link function with 'loam' vs. 'loam with gravel' as a binary dependent variable and a logit link function. Similarly, we examined the risk of nest predation with a generalized linear mixed model using our score of attempted predation (yes vs. no) as a binary dependent variable.

We examined the effects of abiotic conditions on embryos with general linear mixed models using hatching success (%) and the frequency of abnormalities (%). These models also included a binary independent variable that represented whether eggs came from a treatment female or a control female to assess the effects of delayed oviposition. However, origin of eggs did not affect hatching success or the frequency of abnormalities and was removed from the final models. In addition, the interaction of simulated predation x maternal age was not significant in any statistical model and was therefore removed to construct the final models, all of which were evaluated with 2-tailed tests.

## Results

Neither simulated predation nor its interaction with maternal age affected how far females nested from water (Fig. 1; Table 1), forest, or the nearest of the two environmental edges (Table 2). However, old females nested 11 m farther from water on average than young females (29.3 m vs. 18.3 m; Fig. 1; Table 1). Females returned to nest an average of 44.2 h ( $\pm 5.8$  SE; range 0.75–121.5) after their first nest attempt and nested 112.9 m ( $\pm 27.1$ ; range 2.5–653) away from their first nest site. Neither time nor distance between nesting attempts were associated with each other ( $F_{1, 27} = 0.00$ ,  $P = 0.98$ ) or maternal age (both  $P \geq 0.33$ ). In addition, maternal ID explained a significant amount of variation in how far females nested from water (Table 1), but not from the forest edge or nearest of the two (Table 2). The soil type in which females nested was not affected by simulated predation, maternal age, their interaction, or maternal ID (Table 2). Also, 11/30 (37%) females had been captured by our lab group in previous years. However, being captured previously had no effect on how females responded to simulated predation

(distance to water,  $F_{1, 28} = 1.91$ ,  $P = 0.18$ ; distance to forest,  $F_{1, 28} = 0.02$ ,  $P = 0.88$ ; distance to nearest water or forest edge,  $F_{1, 28} = 0.15$ ,  $P = 0.71$ ; soil type,  $F_{1, 28} = 0.15$ ,  $P = 0.70$ ).

Nest sites chosen after simulated predation were 17% more likely to be depredated than nest sites chosen before simulated predation, and this effect was similar for both young and old females (Fig. 2; Table 1). Although maternal age did not predict likelihood of nest predation, maternal ID did (Table 1). Neither hatching success (65% before simulated predation vs. 70% after; Table 1) nor hatchling abnormality frequency (15% before simulated predation vs. 12% after; Table 2) were affected by simulated predation, maternal age, their interaction, or maternal ID. Control clutches did not differ meaningfully from experimental clutches in hatching success (63% vs. 72%, respectively;  $F_{1, 84} = 2.13$ ,  $P = 0.15$ ) or abnormality frequency (11% vs. 15%, respectively;  $F_{1, 66} = 1.13$ ,  $P = 0.29$ ).

No thermal variable was associated with simulated predation, maternal age, their interaction, or maternal ID (Table 2).

## Discussion

Mothers increase their fitness by ovipositing embryos in environments that enhance offspring survival. However, some nesting locations may be risky for female survival (e.g., outside of home range or in a different habitat type). For species with long reproductive lifespans, females should balance reproductive investment with their own survival and future reproduction. However, whether such parent-offspring conflict exists over oviposition choice, and whether females balance those conflicts differently across age, is unclear. We simulated predation attempts on nesting *Chrysemys picta* and predicted females would oviposit closer to the safety of water on their next nesting event and that such nest-site variation would reduce

offspring survival. In contrast, neither young nor old females altered the distance nests were laid from water in response to simulated predation. However, nest sites chosen after simulated predation were 17% more likely to be depredated than nest sites chosen prior to simulated predation. These findings suggest the distance females are willing to travel to oviposit is robust to recent predator exposure, but females may be compromising nest site choice in a way that increases nest predation.

#### *Female response to simulated predation*

Female *C. picta* flee to water after a threat, such as a human, is encountered during terrestrial nesting forays (Delaney et al. 2017). Thus, we predicted females would oviposit closer to the perceived safety of water after simulated predation to reduce their predation risk while nesting. Furthermore, we predicted that such a shift in nest-site choice would be the strongest in young females because they have more future reproductive opportunities at risk compared to older females. However, we found no evidence that females of either age category altered the distance nests were laid from water in response to simulated predation. Additionally, simulated predation did not alter the distance nests were laid from the forest edge or from the nearest water or forest edge. In contrast, Murray River turtle (*Emydura macquarii*) nests were farther from shore following the removal of red foxes (*Vulpes vulpes*), which depredate adults (Spencer 2002). Given that turtles have a relatively long generation time, the change in nest-site choice in *Emydura* was likely a plastic response due to lower perceived risk rather than a response from relaxed natural selection on female preference for distance to water. This issue is not limited to turtles. For example, three-spined sticklebacks (*Gasterosteus aculeatus*) also alter nest-site

choice in the presence of predators by nesting in more structurally complex habitats (Candolin and Voigt 1998).

Despite the lack of a response in our study, older females nested almost twice as far from water as young females, which is consistent with previous work (Harms et al. 2005). Female *C. picta* can move at least 1,284 m between ponds with no effect of body size on distance travelled (House et al. 2010), suggesting size-biased locomotor performance is an unlikely explanation for variation in distance to water in our study (max = 74 m). Older females likely nest farther from water to maximize nest survival (Kolbe and Janzen 2002; Spencer, 2002; Strickland et al. 2010) and accept higher risk because they have lower future reproductive potential compared to younger females (for similar discussion, see Harms et al. 2005; Paitz et al. 2007; Refsnider et al. 2015). Indeed, smaller (and likely younger) red-eared sliders (*Trachemys scripta*) have higher mortality during terrestrial nesting excursions than larger ones (Tucker et al. 1999), suggesting size-biased female risk may contribute to variation in nest distance to water. Females are not likely to improve nest distance to water due to experience because females return to water after nest construction and do not know the fates of their nests. Another possible explanation is that selection removes females that nest close to shore, such that only turtles that nest farther from water remain at older ages. However, females that nest farther from water should have higher risk than those that nest closer to the safety of shore, and longitudinal analyses show that individuals construct nests farther from water with age (Delaney, Hoekstra, and Janzen, in review). Lastly, older turtles produce larger embryos (Harms et al. 2005), which have enhanced dispersal ability (Janzen 1993; Tucker 2000, Janzen et al. 2000a, 2000b; Janzen et al. 2007, Paitz et al. 2007) that may enable their mothers to nest farther from water (Delaney and Janzen 2019).

Thus, the optimal distance to water for nesting is likely a complex balance of risk to nests, future reproductive potential, risk to females, and offspring dispersal ability.

All females retracted into their shells and/or attempted to flee during simulated predation and fled to water shortly after release. Moreover, handling methodology similar to that used in our study stimulates a substantive physiological stress response in *C. picta* (Polich 2016), although such does not necessarily indicate elevated perceived risk. Nevertheless, that females did not consider the human observer as a potential threat is unlikely (see also Bateman et al. 2014). Although turtles did not nest closer to shore following simulated predation, females may have mitigated risk by nesting at a location away from the predator encounter (up to 653 m) or returning later (up to 5 days). Indeed, many animals increase space between predators (Sih 1982; Hammond et al. 2007; Breed et al. 2017; Delaney and Warner 2016, 2017a, 2017b) or alter activity time (Lima 1988; Skelly and Werner 1990; Wooster and Sih 1995; Creel et al. 2017) to reduce risk. This study was conducted in an area with extensive human recreational use. Thus, females may have perceived the area as high risk at the outset during their first nesting attempt. If females were already nesting with high perceived risk, our ability to observe a further response may have been impeded. Regardless, disturbance during simulated predation should have been perceived as an escalation of risk because turtle handling by recreationists is relatively rare at this site despite high human activity. An alternative explanation is that turtles at this site have acclimated to humans and perceive their presence as low or no risk (see Bateman et al. 2014). In support of this possibility, the presence of recreationists does not affect the number of turtles that emerge from water to nest (Bowen and Janzen 2008). Additionally, larger individuals at this site have a lower corticosterone response to human handling than smaller turtles, suggesting turtles may acclimate to human disturbance with age (Polich 2016; see above for alternative

explanations). Future work that addresses these questions at sites with lower human activity would be enlightening.

### *Consequences for offspring*

Nest sites chosen after simulated predation were more likely to be depredated than nest sites chosen before simulated predation. This effect occurred despite no change in the distance nests were laid from water. This outcome suggests females chose different microenvironments after simulated predation in a way we did not quantify. Perhaps nest sites varied in distance to anthropogenic structures, which can reduce the probability of nest predation (Strickland and Janzen 2010). We quantified soil type as ‘loam’ or ‘loam with gravel’, but finer resolution might have explained the variation in predation rates observed in our study. Indeed, soil composition affects nest predation rates at this site (Hoekstra et al., unpublished). An alternative explanation is that female scent deposited near second nest sites was more recent and attractive to predators than scent deposition at the first nest attempt site. However, to reduce the likelihood that scent variation between nest sites would influence predation risk, we placed artificial nests about 0.75 m adjacent to the original locations that females chose. In addition, predation rate was not correlated with time since a female nested or attempted to nest at that site ( $F_{1, 26} = 0.44$ ,  $P = 0.51$ ). Nevertheless, predation risk also influences nest-site choice in other systems. For example, multiple bird species nest in safer habitats when nest predators are near (Eggers et al. 2006; Forstmeier and Weiss 2004; Fontaine and Martin 2006). However, evidence of females altering nest-site choice because of predation risk to themselves is rare. As mentioned above, Murray River turtles (Spencer 2002) and three-spined sticklebacks (Candolin and Voigt 1998) nest in habitats that reduce risk to adults when predators are present. Moreover, in the case of Murray



River turtles, this nest-site variation costs offspring survival, suggesting parents balance risk to themselves with risk to offspring (Spencer 2002). We add another example and show that *C. picta* also nest in locations with higher nest-predation risk when adults experience risk to themselves.

No thermal variable, average hatching success, or abnormality frequency were affected by simulated predation. Thus, although females may have chosen nest sites with higher predation risk after perceiving risk to themselves, they still chose microclimates that were adequate for normal embryonic development. Control and experimental clutches did not differ in hatching success or abnormality frequency, which suggests that delaying oviposition alone did not exert deleterious effects on offspring development. Embryos of *C. picta* develop into late gastrulae in the oviduct and then arrest development until oviposition (Ewert 1985). Such developmental arrest may enable maternal plasticity in oviposition timing, but embryos develop abnormally if they are experimentally retained too long (Cunningham 1923; Risley 1944; Ewert 1985). Similarly, chickens (*Gallus gallus*) that delay oviposition (up to 1 d) because of stress produce eggs with a higher degree and frequency of abnormal shells, which can have negative effects on offspring development (Reynard and Savory 1999). In contrast, fence lizards (*Sceloporus undulatus*) that retain eggs longer than normal produce offspring that are heavier and farther along development at oviposition and have higher post-hatching survival (Warner and Andrews 2003). We also found no consequences for delaying oviposition and show that oviposition timing in wild *C. picta* is highly plastic (up to 5 d).

*Other considerations of female behaviour*

Although females did not nest closer to water after simulated predation, they may have altered nesting behaviour in aspects we did not quantify. Females may have rushed searching for or constructing nests because they had already delayed oviposition and perceived risk was elevated. For example, a large rock prevented one female from constructing a normal-sized nest cavity, and she only laid four eggs (average clutch size at this site is  $10.5 \pm 2.0$  SD (Morjan 2003)). Similarly, another female laid eggs in a cavity containing a large rock (about 1/5 of cavity) and then destroyed multiple eggs while covering them with soil. Both females may have improved their nesting behaviour if they had not previously delayed oviposition or because they were rushing to return to water because of perceived risk. In support of the latter explanation, another female constructed a typical nest and oviposited but did not cover her eggs with soil before returning to water. Thus, future work that quantifies time invested into searching for and constructing nests (e.g., Congdon and Gatten 1989; Frye et al. 2017) may further assess the effects of perceived risk on nesting behaviour.

Maternal ID explained considerable variation in the distance nests were laid from water and the likelihood of nest predation. Because repeatability sets an upper bound for the heritability of a trait (Boake 1989), finding that maternal ID explained variation in distance to water suggests this trait may respond evolutionarily to selection. Indeed, other nesting behaviours are repeatable (canopy cover, Janzen and Morjan 2001; Valenzuela and Janzen 2001; McGaugh et al. 2010; geographic site, Valenzuela and Janzen 2001; phenology, McGaugh et al. 2010) and heritable (phenology and canopy cover, McGaugh et al. 2010) in *C. picta* (reviewed in Janzen et al. 2019). Finding that maternal ID was a significant predictor of predation likelihood suggests the consequences of nest-site choice are also repeatable. Thus, although we found no

plastic response to simulated predation, selection may shape female preference for distance to water as an age-related reaction norm.

### *Conclusion*

Mothers of oviparous species increase their fitness by nesting in locations that enhance offspring survival and phenotype. However, long-lived species must also nest in locations that facilitate female survival. Moreover, this tradeoff should shift towards heavier reproductive investment as females age and future reproductive opportunities decrease. However, we found no evidence that female *C. picta* of any age nest closer to the safety of water following simulated predation. Despite this lack of a response, nest sites chosen after simulated predation were more likely to be depredated than those chosen before simulated predation, suggesting females altered nest-site choice in a way we did not quantify. We provide a rare example that predation risk to adult animals can influence nest-site choice and, moreover, that such a shift affects the probability of nest predation and, hence, offspring survival. And although we found no effect of age on responses to perceived risk, we present a scarce examination of how plastic investment tradeoffs might vary across reproductive life. Future work that occurs at less human-disturbed sites or that quantifies maternal effort invested in searching for and constructing nests will further our understanding of how predation risk affects maternal investment and influences fitness.

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**Table 1** Statistical results of focal models testing the effects of simulated predation, maternal age, and their interaction on *Chrysemys picta* maternal behaviour and offspring consequence. Significant effects are bolded. \*The interaction of simulated predation x maternal age was not significant in any model and was removed to construct the final models.

Dependent variable	Independent variables			Random effect
	Simulated predation	Maternal age	Simulated predation x maternal age	Maternal ID
Distance to water	F <sub>1, 29</sub> =0.67 P=0.414	F <sub>1, 29</sub> =3.95 <b>P=0.047</b>	*F <sub>1, 28</sub> =0.90 P=0.350	χ <sup>2</sup> =5.99 <b>P=0.016</b>
Nest predation	F <sub>1, 29</sub> =6.48 <b>P=0.011</b>	F <sub>1, 29</sub> =0.05 P=0.827	*F <sub>1, 28</sub> =0.40 P=0.530	χ <sup>2</sup> =5.33 <b>P=0.021</b>
Hatching success	F <sub>1, 27</sub> =0.02 P=0.889	F <sub>1, 27</sub> =0.10 P=0.757	*F <sub>1, 26</sub> =0.22 P=0.639	χ <sup>2</sup> =0.67 P=0.415

**Table 2** Statistical results of secondary interest models testing the effects of simulated predation, maternal age, and their interaction on *Chrysemys picta* maternal behaviour and offspring consequence. \*The interaction of simulated predation x maternal age was not significant in any model and was removed to construct the final models.

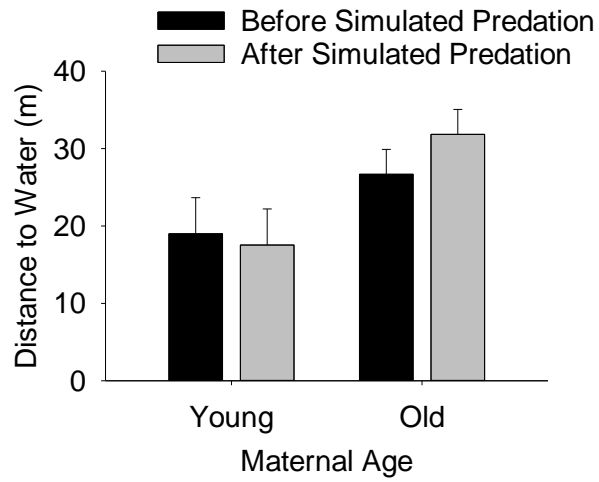
Dependent variable	Independent variables			Random effect
	Simulated predation	Maternal age	Simulated predation x maternal age	Maternal ID
Dist. to forest	F <sub>1, 29</sub> =0.05 P=0.823	F <sub>1, 29</sub> =1.21 P=0.271	*F <sub>1, 28</sub> =0.43 P=0.512	χ <sup>2</sup> =2.38 P=0.123
Dist. to edge	F <sub>1, 29</sub> =0.39 P=0.531	F <sub>1, 29</sub> =1.86 P=0.173	*F <sub>1, 28</sub> =0.27 P=0.603	χ <sup>2</sup> =3.38 P=0.066
Soil type	F <sub>1, 29</sub> =0.89 P=0.345	F <sub>1, 29</sub> =0.00 P=0.992	*F <sub>1, 28</sub> =0.00 P=0.947	χ <sup>2</sup> =2.12 P=0.145
Thermal PC1	F <sub>1, 28</sub> =1.79 P=0.181	F <sub>1, 28</sub> =0.35 P=0.555	*F <sub>1, 27</sub> =0.49 P=0.480	χ <sup>2</sup> =0.50 P=0.480
Thermal PC2	F <sub>1, 28</sub> =0.03 P=0.869	F <sub>1, 28</sub> =3.46 P=0.063	*F <sub>1, 27</sub> =0.32 P=0.570	χ <sup>2</sup> =2.84 P=0.092
Thermal PC3	F <sub>1, 28</sub> =1.04 P=0.307	F <sub>1, 28</sub> =0.07 P=0.787	*F <sub>1, 27</sub> =0.37 P=0.544	χ <sup>2</sup> =0.57 P=0.450
CTE during TSP	F <sub>1, 28</sub> =1.43 P=0.233	F <sub>1, 28</sub> =1.09 P=0.297	*F <sub>1, 27</sub> =0.49 P=0.486	χ <sup>2</sup> =0.57 P=0.450
Abnormalities	F <sub>1, 21</sub> =0.87 P=0.351	F <sub>1, 21</sub> =1.44 P=0.229	*F <sub>1, 20</sub> =0.08 P=0.780	χ <sup>2</sup> =0.90 P=0.342

**Supplementary Table 1** Correlations of day of year, thermal variables, CTE (constant temperature equivalent), canopy openness, and transmitted solar radiation experienced by *Chrysemys picta* nest sites.

	Minimum temp.	Maximum temp.	Average temp.	CTE	Canopy openness	Solar radiation
Day of year	$r = 0.73, P < 0.01$	$r = -0.28, P = 0.03$	$r = 0.15, P = 0.25$	$r = -0.02, P = 0.86$	$r = 0.27, P = 0.04$	$r = 0.19, P = 0.14$
Minimum temp.		$r = -0.24, P = 0.06$	$r = 0.24, P = 0.06$	$r = -0.04, P = 0.78$	$r = 0.14, P = 0.31$	$r = 0.16, P = 0.22$
Maximum temp.			$r = 0.80, P < 0.01$	$r = 0.91, P < 0.01$	$r = 0.15, P = 0.27$	$r = 0.35, P < 0.01$
Average temp.				$r = 0.90, P < 0.01$	$r = 0.36, P < 0.01$	$r = 0.52, P < 0.01$
CTE					$r = 0.31, P = 0.02$	$r = 0.52, P < 0.01$
Canopy openness						$r = 0.85, P < 0.01$

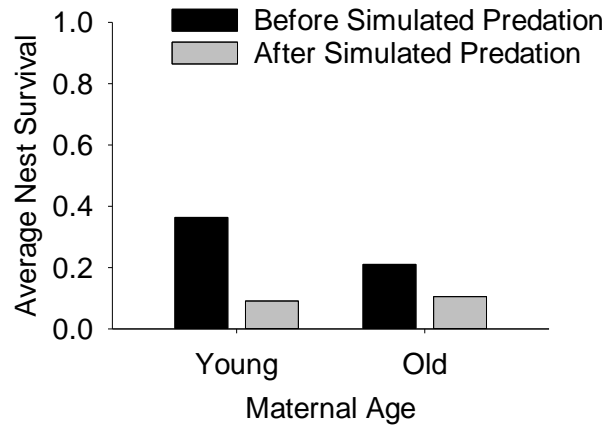
**Supplementary Table 2** Principal component loadings for thermal variables of*Chrysemys picta* nests.

Variables	PC1	PC2	PC3
Average temperature	-0.53	-0.21	0.43
Minimum temperature	-0.11	0.62	0.70
Maximum temperature	-0.43	-0.60	0.16
Canopy openness	-0.46	0.39	-0.46
Solar radiation	-0.55	0.23	-0.29
Variation explained	51%	26%	19%



**Figure 1** Effects of simulated predation and maternal age on how far *Chrysemys picta* nested from water. Data are plotted as least-squares means  $\pm$  1 standard error. Statistical results are reported in Table 1.





**Figure 2** Effects of simulated predation and maternal age on the likelihood of predation of nest sites chosen by *Chrysemys picta*. Statistical results are reported in Table 1.

### CHAPTER 3. OFFSPRING DISPERSAL ABILITY COVARIES WITH NEST-SITE CHOICE

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#### Abstract

Optimal maternal investment is often a tradeoff between conflicting pressures and varies depending upon environmental context and intrinsic female traits. Yet, offspring phenotype might also interact with such factors to influence investment. In aquatic turtles, terrestrial nests constructed farther from shore often have higher survival because nest predators tend to forage along environmental edges. However, offspring from eggs deposited farther inland must migrate farther to water upon emergence. We released hatchling common snapping turtles (*Chelydra serpentina*) at varying distances from a drift fence and monitored survival during overland dispersal. Survival decreased with dispersal distance and no selection on body size was evident for hatchlings dispersing from short- or intermediate-distances. However, survival increased with body size for hatchlings dispersing from the longest distance. Moreover, females producing larger and better dispersing offspring oviposited farther from water than females that produced smaller and poorer dispersing offspring. This conditional (on offspring body size) tradeoff

suggests female investment can be sensitive to offspring phenotype and that such covariation between nest-site choice and offspring dispersal ability can maximize offspring survival and, thus, maternal fitness. Future work that considers the role of offspring performance on maternal behavior will elucidate an underappreciated influence of investment strategies.

### **Introduction**

Mothers increase their fitness by investing resources in offspring (Trivers 1972). However, resource limitation or conflicting pressures may generate maternal investment tradeoffs (Stearns 1989, 1992; Roff 1992; Balme et al. 2017; Wiernasz and Cole 2018). The optima of such tradeoffs can shift depending upon environmental context (e.g., predation risk, Ghalambor and Martin 2001; Fontaine and Martin 2006; Taborsky 2006; Segers and Taborsky 2011; social setting, Russell et al. 2007; Taborsky et al. 2007) or female traits (e.g., body condition or age; Monaghan et al. 1998; Velando et al. 2006; Kindsvater et al. 2010; Arnold et al. 2018). Yet, optimal investment may also depend upon offspring phenotype. For example, various animals differentially invest in sons versus daughters because parents can increase their fitness by investing in the sex with the greatest return (e.g., Altmann and Samuels 1992; Olsson and Shine 2001; Spelt and Pichegru 2017). Although offspring phenotype can affect post-natal food provisioning in birds and mammals (Magrath 1990; Price and Ydenberg 1995; Wells 2003; Middleton et al. 2007; Soley et al. 2011; Merklings et al. 2014), the role of offspring phenotype on pre-natal investment is less understood (but see offspring size vs number tradeoffs, Smith and Fretwell 1974; Einum and Fleming 2000; Janzen and Warner 2009). In addition, whether offspring performance traits might influence maternal investment strategies is unknown (*sensu* Sinervo 1990).

For oviparous animals, the location that mothers choose to nest is often the greatest determinant of early-life success for offspring (Bernardo 1996; Resetarits 1996). For example, nest-site choice can influence offspring phenotype (Janzen 1994; Shine et al. 1997), predation risk (Spencer and Thompson 2003; Forstmeier and Weiss 2004), and proximity to suitable juvenile habitat (reviewed in Refsnider and Janzen 2010). Although environmental conditions and female traits influence nest-site choice, few studies have examined if females choose nest sites that are specifically tailored for the phenotypes of their offspring. Moreover, the few tests of adaptive nest-site choice as a function of offspring phenotype found that females selected nest sites that were as equally beneficial for other offspring as their own (Shine et al. 1997; Mitchell et al. 2013). Nevertheless, offspring phenotype and nest-site choice may still covary if adaptive. For example, embryos that develop into good dispersers could be deposited farther from juvenile habitat if doing so is advantageous (e.g., reduce predation risk of nest). In contrast, embryos that develop into poor dispersing offspring could constrain females to nest closer to juvenile habitat and experience elevated nest predation risk. Thus, while rare, studies that consider the potential impact of offspring phenotype on nest-site choice may reveal an important influence on fundamental investment behavior.

Freshwater turtles are well suited to address this major conceptual issue in behavioral ecology. The survival of terrestrial nests increases with distance to water, because nest predators (e.g., raccoons) often forage along environmental edges (Temple 1987; Kolbe and Janzen 2002a; Spencer 2002; Marchand and Litvaitis 2004; Strickland et al. 2010). However, offspring from eggs deposited farther inland must migrate a greater distance to water upon emergence from their nests. Thus, females may balance a tradeoff between nesting far from water to increase nest survival with nesting close to water to reduce offspring dispersal distance. Furthermore,

mortality during these early life stages is high in aquatic turtles (e.g., *Chelydra serpentina*, 41.1% Janzen 1993; 33–41% Congdon et al. 1999; 37.1% Kolbe and Janzen 2001; *Chrysemys picta*, 25% Tucker 2000, 22% Mitchell et al. 2013; *Trachemys scripta*, 66% Janzen et al. 2000a; 65.1% Janzen et al. 2000b; 42.9% Tucker 2000; reviewed in Iverson 1991), suggesting selection on maternal investment strategies is considerable. Indeed, selection often favors larger hatchlings during dispersal to water (Janzen 1993; Tucker 2000; Janzen et al. 2000a, 2000b; Janzen et al. 2007; Paitz et al. 2007). This trend likely results from survival declining the longer hatchlings spend dispersing (Janzen et al. 2007) and larger offspring dispersing faster (Janzen et al. 2000a, 2000b; Paitz et al. 2007). In addition, older and larger females oviposit larger eggs (Congdon and Gibbons 1985; Congdon et al. 1987; Bowden et al. 2004) and do so farther from water (Harms et al. 2005). Thus, females that oviposit larger eggs may do so farther from water because their larger offspring are capable of longer dispersal (Fig. 1A) and predation on nests decreases with distance from water (Fig. 1B).

To assess the role of offspring dispersal ability in maternal investment strategies, we conducted a dispersal experiment with 428 hatchling common snapping turtles (*Chelydra serpentina*) from 15 nests. We released hatchlings at three distances from water typical of natural nests and subsequently monitored survival and time to disperse during overland dispersal. We predicted larger hatchlings would have higher survival and disperse faster than smaller hatchlings. Furthermore, we predicted these effects would be stronger when offspring were required to disperse farther to water (Fig. 1A). Prior to collection for the dispersal experiment, we also measured how far nests were naturally constructed from water. Because females may tailor nesting strategy to their young's dispersal ability, we predicted offspring from natural nests

located farther from water would have better dispersal performance in our experiment than offspring from nests closer to shore.

## Methods

We monitored the nesting behavior of 16 *C. serpentina* at the Thomson Sand Prairie along the Mississippi River in Illinois from 26 May to 3 June 2017. After a nesting event concluded, we measured the distance between the nest and the River with a GPS (down to  $\pm 2.4$  m accuracy, Garmin eTrex 20). We then excavated eggs ( $n = 16\text{--}85$  per nest) and moved them to an artificial nest block protected with wire mesh until hatching. No eggs were damaged during this excavation and burial process. Moreover, predation on natural, unprotected nests is high at this site (65%, Kolbe and Janzen 2002b), yet we only lost 1 nest (6%) to predation in our protected nest block. Within the nest block, we placed each clutch about 21 cm deep (near the average depth ( $\sim 18$  cm) and within the range (up to 21.5 cm) at our site; Kolbe and Janzen 2002b; Telemeco et al. 2016) and 0.5 m from the nearest other artificial nest. Incubation in this common-garden arrangement in the field reduced variation in incubation environments among clutches while still exposing embryos to natural abiotic conditions in a location often used for nesting. We placed iButton data loggers in the middle ( $\sim 16$ -cm deep) of three nests to monitor thermal conditions in the artificial nest block. We analyzed nest temperatures from the day the last clutch was placed in the nest block (7 June) to the day of first emergence from a nest (31 July), which represents 77–90% of the entire developmental period of experimental nests.

We encircled nests with 15-cm high PVC on 25 July and monitored nests twice daily for hatchling emergence. The PVC contained emerging turtles from a nest, enabling us to assign clutch to each hatchling. After emergence, we weighed hatchlings to the nearest 0.01 g and

measured straight carapace length (SCL) to the nearest 0.01 mm. We notched either the left or right 11th marginal scute and photographed the plastrons to uniquely identify hatchlings upon recapture. The marginal scute mark allowed us to verify that a recaptured hatchling was from our experimental release and reduced the number of photographs we needed to survey by half (sensu Janzen 1993). We housed hatchlings at the clutch level in covered plastic containers (up to 30 hatchlings per container; container size = 23 cm x 35 cm x 9 cm) placed in a large cooler (mean =  $4.4 \pm 2.22$  SD, range = 2–9 days), which we kept in the shade at the field site to reduce metabolic activity until enough hatchlings emerged for the dispersal experiment to begin. We monitored captive hatchlings at least twice daily to verify adequate thermal and moisture conditions. We observed no aggression among hatchlings and provided no food, but sprayed clean water into each container daily. No hatchlings died in captivity, and length of time in captivity did not affect post-release survival ( $F_{1, 412} = 0.92$ ;  $P = 0.3373$ ), suggesting our processing and housing methodology did not adversely affect hatchlings.

We constructed a straight 250 m drift fence that paralleled the Mississippi River and buried 4.5-liter plastic jars every 5 m to capture hatchlings as they dispersed from their terrestrial release locations to water (Fig. S1; sensu Janzen 1993; Congdon et al. 1999; Kolbe and Janzen 2001). We randomly divided up to 30 hatchlings/clutch into six groups and released them at either 25, 62.5, or 100 m from the fence. Each release distance was spatially replicated twice so that each release distance had between 69 and 73 dispersers (total  $N = 428$ ), which falls within the natural clutch size range of *C. serpentina* (Iverson et al. 1997; Kolbe and Janzen 2001; Ernst and Lovich 2009). Thus, there were two groups of release points (i.e., a North and a South replicate) with a release point for each distance from water. On 8 August, we excavated 15 cm pits at each release point to simulate natural nests, inserted hatchlings into the pits, and placed upturned 19-liter

buckets over the release points. We allowed hatchlings to acclimate for 15 minutes and then used 10 m long ropes to remove buckets to reduce disturbance by observer presence. Peak nest emergence of *C. serpentina* occurs from 1000–1100 h in Michigan (Congdon et al. 1999), and most terrestrial movement occurs after sunrise and before 1300 h at our study site (Janzen 1993; Kolbe and Janzen 2002c). Thus, we released hatchlings at 1000 hours and did not re-enter the dispersal area until the experiment concluded on 16 August. We checked pitfall traps at 0700, 1300, and 1900 h each day by walking along the river side of the fence to minimize disturbance by the observer. For hatchlings that reached the fence, we recorded the time it took hatchlings to disperse (= dispersal time) and the distance between the closest spot on the fence for that hatchling's release point and the trap the hatchling was caught in (= dispersion along fence). We checked traps for 9 days after release at which point the recapture rates were very low (Fig. S2) and scored all hatchlings not recaptured as dead. Forty-seven percent (203/428) of hatchlings were not recovered, which is comparable to dispersal mortality in other studies of aquatic turtles (discussed above). We released all recaptured hatchlings in the River after identification. This work adhered to ABS/ASAB guidelines for ethical treatment of animals and was approved by the Iowa State University Institutional Animal Care and Use Committee (5-17-8509-J).

### *Analysis*

We ran all analyses with SAS software (version 9.4). All mixed models included clutch as a random effect. We evaluated random effects using likelihood-ratio tests. To examine how offspring size and release distance influenced survival during dispersal, we first ran a generalized linear mixed model with survival to fence as a binary dependent variable and body size, release distance, their interaction, and replicate as independent variables. Offspring mass and SCL were



highly correlated ( $r^2 = 0.76$ ;  $P < 0.0001$ ); therefore, future analyses focused mainly on mass as the metric for offspring body size unless otherwise stated. The body size\*release distance interaction was not significant and was removed from the final model. We chose to analyze survival with generalized linear mixed models instead of other methods because (1) the fate of every individual was considered known, (2) we could not determine when hatchlings perished, and (3) we wanted to account for clutch ID as a random effect. We also calculated average linear and quadratic selection gradients for offspring mass and SCL at each release distance with logistic regressions to quantify the relative importance of body size on survival (Janzen and Stern 1998). We ran independent analyses for mass and SCL because they were correlated. We detected no significant quadratic selection, so we removed quadratic terms from the final models.

We used general linear mixed models to assess the effects of offspring body size and release distance on dispersal time (time-to-fence in days) and dispersion along the fence (distance between the closest spot on the fence for a hatchling's release point and the trap the hatchling was caught in) as the dependent variables. Offspring mass, release distance, their interaction, and replicate were the independent variables. The offspring mass\*release distance interaction was not significant in either analysis and was removed from the final models.

We also employed linear regression to assess if offspring dispersal ability was related to how far females nested from water naturally. We regressed the collective survival (%) of clutch mates released at 25, 62.5, and 100 m, as well as average offspring mass, on how far females oviposited from water. We ran all four regressions separately because independent variables were correlated. One female crossed two roads and nested much farther from water (185 m) than the rest ( $51.3 \pm 5.61$  m); therefore, we excluded her data from these regression analyses. These regressions were 1-tailed tests because our predicted effects were directional.

## Results

Incubating embryos experienced mean, minimum, and maximum nest temperatures  $\pm$  SD on the order of  $28.9^{\circ}\text{C} \pm 0.39$ ,  $23.2^{\circ}\text{C} \pm 0.29$ , and  $34.8^{\circ}\text{C} \pm 1.04$ , respectively. These thermal conditions are similar to thermal conditions experienced by natural nests at this field site (range  $26.3\text{--}34.1^{\circ}\text{C}$ , Kolbe and Janzen 2001; range of means  $\sim 23.4\text{--}30.5^{\circ}\text{C}$ , St. Juliana et al. 2004). During the 9-day dispersal period, hatchlings experienced minimum daily air temperatures of  $15.7^{\circ}\text{C} \pm 1.62$  SD (range  $13\text{--}18^{\circ}\text{C}$ ) and maximum daily air temperatures of  $25.9^{\circ}\text{C} \pm 1.76$  SD (range  $23\text{--}28^{\circ}\text{C}$ ; data gathered from a weather station  $\sim 15$  km away, [www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)). The only precipitation occurred on the fourth and eighth days of dispersal, with 0.38 and 0.10 cm of rainfall, respectively.

Potential predators observed in the dispersal area included one opossum (*Didelphis virginiana*) and one hognose snake (*Heterodon nasicus*). However, we detected fresh tracks of coyotes (*Canis latrans*) and raccoons (*Procyon lotor*), and have previously noted striped skunks (*Mephitis mephitis*), red foxes (*Vulpes vulpes*), various raptors, American crows (*Corvus brachyrhynchos*), blue jays (*Cyanocitta cristata*), great blue herons (*Ardea herodias*), common egrets (*Ardea alba*), bullsnakes (*Pituophis catenifer*), blue racers (*Coluber constrictor*), and northern water snakes (*Nerodia sipedon*) nearby. We did not observe predator tracks or disturbance around collection pits, suggesting predators did not consume hatchlings captured in traps.

Average egg mass of clutches was positively correlated with average offspring mass at emergence from nests ( $r = 0.91$ ,  $P < 0.0001$ ), but neither variable was correlated with clutch size (egg mass,  $r = 0.05$ ,  $P = 0.8700$ ; offspring mass,  $r = 0.11$ ,  $P = 0.7289$ ). The overall average egg

mass, offspring mass, SCL, and clutch size  $\pm$  SD were  $14.39 \pm 1.67$  g,  $11.40 \pm 1.32$  g,  $31.35 \pm 1.59$  mm, and  $43 \pm 14$ , respectively.

Offspring survival decreased with increasing release distances (Fig. 2A; Table 1). The generalized linear mixed model did not detect an effect of hatchling mass or an interaction of mass\*release distance on survival (Table 1). Field studies should use >200 individuals for adequate power to detect selection (Hersch and Phillips 2004), yet we used 141–145 hatchlings per release distance. Thus, sample size at each release distance may have precluded the ability of the model to detect a mass\*release distance interaction. However, selection gradient analyses revealed mass and release distance interactively affected survival (Table 2). Neither SCL nor body mass influenced offspring survival at 25 or 62.5 m, but longer and heavier offspring had higher survival than shorter and lighter offspring when initiating dispersal 100 m from the fence (Fig. 3; Table 2). To illustrate, hatchlings smaller than the mean averaged 2–3 times lower probability of survival than their larger counterparts at the greatest distance (Fig. 3). We found no evidence of quadratic selection on either SCL or mass for hatchlings dispersing from any distance (Table 2). Offspring from the northern replicates (47.5%) had lower survival than those from the southern replicates (57.9%; Table 1). Clutch ID did not explain a substantive amount of variation in offspring survival ( $\chi^2 = 1.50$ ,  $P = 0.1104$ ).

Dispersal time increased with release distance (Fig. 2B; Table 1). Offspring mass, its interaction with release distance, release replicate (Table 1), and clutch ( $\chi^2 = 2.32$ ,  $P = 0.3127$ ) did not explain a meaningful amount of variation in dispersal time.

Dispersion along the fence increased with release distance (Fig. 2C; Table 1). However, neither body mass nor its interaction with release distance affected dispersion (Table 1). Hatchlings from the northern release points ( $27.2 \pm 2.04$  m) dispersed more directly to the fence

than those from the southern replicates ( $33.7 \pm 1.95$  m; Table 1). Clutch again was not an important predictor of variation in dispersion ( $\chi^2 = 1.66$ ,  $P = 0.4363$ ). Most hatchlings were caught in pitfalls along the center of the drift fence and no hatchlings were caught in pitfalls along the terminal 25 m of either end of the fence (Fig. S3).

Females that produced offspring with higher survival when dispersing from 100 m constructed nests farther from water than females that produced offspring with lower survival when dispersing from 100 m (Fig. 4C; Table 1). The survival estimates of offspring dispersing from 25 and 62.5 m were not correlated with how far females oviposited from water (Fig. 4; Table 1). In addition, females that produced heavier offspring constructed nests farther from water than females that produced lighter offspring (Fig. 4D; Table 1).

## Discussion

Optimal maternal investment in offspring is often a balance of conflicting pressures. While some maternal investment tradeoffs are well studied (e.g., offspring size vs. clutch size), how offspring performance might affect pre-natal investment strategies is unknown. We found that larger hatchling turtles had higher survival during dispersal from long distances than did smaller offspring. Moreover, mothers that produced clutches that were better at dispersing long distances accordingly oviposited their clutches farther from water. These findings suggest mothers producing good dispersers maximize the benefits of reduced nest predation farther from water, whereas mothers producing poor dispersers nest closer to shore because their offspring are less capable of dispersing longer distances.

In our experiment, juveniles released farther from the drift fence experienced lower survival than those released closer to the fence. Increasing mortality with dispersal distance is

often assumed for dispersing animals (Brooker et al. 1999; Refsnider and Janzen 2010; Bonte et al. 2011), but empirical evidence is lacking. Previous studies of freshwater turtles found no effect of increasing dispersal distance on offspring survival (*C. serpentina*, Congdon et al. 1999; *Chrysemys picta*, Paitz et al. 2007) or a minor effect opposite of predictions (61% survival from 35 m and 65% survival from 70 m, *C. serpentina*, Kolbe and Janzen 2001). We increased the variation in release distances compared to previous studies to encompass more of the natural range of dispersal distances that wild *C. serpentina* experience, which presumably enabled us to detect an effect of dispersal distance on survival. Nevertheless, animals that disperse farther are likely exposed to predators and unfavorable environments for longer durations, which may explain the higher mortality observed in our experiment (for similar discussion, see Janzen et al. 2007). In addition, evidence from studies of roughed grouse (*Bonasa umbellus*, Yoder 2004), snowshoe hares (*Lepus americanus*, Sievert and Keith 1985), and *C. serpentina* (Janzen 1995) support theoretical predictions (Lima 1998) that such prolonged movement elevates predation risk. However, to our knowledge, we provide the first empirical support for the assumption that increasing dispersal distance from nests reduces offspring survival in animal taxa.

We predicted larger neonates would have higher survival during dispersal than smaller neonates, and that this size effect would increase with longer dispersal distance. Body size did not affect survival of hatchlings dispersing short or intermediate distances but, in line with predictions, larger hatchlings had higher survival than smaller hatchlings when dispersing the longest distance during our experimental release. Selection generally favors larger turtle offspring during dispersal in *C. serpentina* (Janzen 1993; but see Congdon et al. 1999; Kolbe and Janzen 2001), *C. picta* (Mitchell et al. 2013; Tucker 2000; Paitz et al. 2007), and *Trachemys scripta* (Tucker 2000; Janzen et al. 2000a, 2000b; Janzen et al. 2007; Mitchell et al. 2013; but see

Filoramo and Janzen 2002). For some animals with optional dispersal, larger juveniles are more likely to disperse than smaller juveniles (reviewed in Bowler and Benton 2005), possibly because larger juveniles are better able to tolerate the energetic costs and/or predation risk associated with greater movement. In freshwater turtles, size-biased survival during dispersal may be underpinned by survival decreasing with time exposed on land (Janzen et al. 2007) and larger offspring dispersing faster (Janzen et al. 2000a, 2000b). Nevertheless, we provide novel evidence that offspring size effects on dispersal success can depend upon the distance offspring are born from suitable juvenile habitat. Such crucial interactions between offspring phenotype and nest-site choice should be prime targets of selection driving maternal investment strategies.

Juveniles released farther from the fence arrived at the fence later and dispersed less directly to the fence. We predicted larger hatchlings would disperse faster than smaller hatchlings, as found in other freshwater turtles (*C. picta*, Tucker 2000; Paitz et al. 2007; *T. scripta*, Janzen et al. 2000a, 2000b). However, we found no effect of body size on dispersal time. Despite the importance of body size on locomotor performance in hatchling *C. picta* and *T. scripta*, previous dispersal experiments with *C. serpentina* have detected no effect of body size on dispersal time (Janzen 1993; Congdon et al. 1999; Kolbe and Janzen 2001). Thus, while important for emydids, the effect of body size on dispersal speed may not be ubiquitous across all turtles. Additionally, dispersal speed data were only attainable on hatchlings that survived to the fence (N = 225), and survival at the longest distance was size-biased (discussed above). Thus, the lower survival of smaller hatchlings dispersing from the longest distance may have inhibited our ability to examine how juvenile size might affect dispersal speed.

We considered all hatchlings that did not reach the fence within 8 days after release as dead. Dispersal to the fence was densest around the middle of the fence (most direct path) and

tapered off towards the distal ends of the fence. Indeed, no hatchlings were caught in the terminal 25 m of the fence on either end. Thus, successful dispersal around the fence was unlikely. In addition, 96% of recaptures occurred within 4 days of release, suggesting successful dispersal transpired rapidly after release. Moreover, mortality increases the longer hatchlings are exposed on land (Janzen et al. 2007). Collectively, these results support our assumption that non-captured hatchlings likely perished. Although we did not observe mortality events, avian predation can be heavy on hatchling turtles during dispersal (Janzen et al. 2000b), and we observed raccoon, opossum, and coyote activity around the periphery of the dispersal area. In addition, dispersing turtles may dehydrate, with water loss increasing with time spent on land (Kolbe and Janzen 2002c). Because precipitation only occurred on the fourth and eighth days of release, dehydration might have been a source of mortality during our experiment. Larger hatchling *C. serpentina* have higher rates of evaporative water loss, but should survive longer on land because of greater absolute water content compared to smaller individuals (Finkler 2001). Thus, our finding that selection favored larger hatchlings when dispersing from the longest release distance further suggests dehydration, in addition to predation, as a source of mortality in our study.

Turtle nests constructed farther from environmental edges often experience a reduced likelihood of predation (Kolbe and Janzen 2002a; Marchand and Litvaitis 2004; Strickland et al. 2010). However, for embryos that successfully hatch, neonates must then emerge from the nests and disperse to water. Thus, maternal investment should evince a tradeoff between maximizing nest survival farther from water and reducing dispersal distance closer to shore. As discussed above, larger hatchlings had higher survival than smaller hatchlings during dispersal from the longest distance in our experiment. Therefore, mothers producing these bigger and better dispersing offspring should construct nests farther from water because their offspring are more

capable of dispersing longer distances to water upon emergence. As predicted, mothers that produced heavier offspring and offspring that were better dispersers at long distances constructed nests farther from water than mothers that produced lighter offspring and offspring that were poorer dispersers at long distances. Nesting closer to shore elevates predation risk for embryos in the nest, but if smaller offspring successfully hatch, they need to be close enough to shore to have a reasonable probability of surviving dispersal to water.

These results suggest maternal investment strategy can indeed be sensitive to offspring phenotype. Diamondback terrapin (*Malaclemys terrapin*) that produce larger eggs nest in warmer locations than mothers that produce smaller eggs, and warmer nests are more likely to produce female offspring (Roosenburg 1996). If female offspring benefit more from hatching from larger eggs than male offspring, then such covariation between nest-site choice and offspring size would be adaptive (see also Morjan and Janzen 2003). However, few studies have tested for adaptive nest-site choice dependent upon offspring phenotype. Studies of a lizard (*Bassiana duperreyi*, Shine et al. 1997) and a turtle (*C. picta*, Mitchell et al. 2013) cross-fostered eggs between nests to examine if mothers chose nest sites that were tailored for their offsprings' phenotypes. However, both studies found that mothers chose nest sites that were equally beneficial for unrelated offspring as they were for their own offspring. In contrast, offspring growth of a fly (*Liriomyza sativae*, Via 1986) and a butterfly (*Euphydryas editha*, Singer et al. 1988) on specific host plants increased with female preference for that host plant. Although these studies suggest oviposition choice can covary with offspring traits, the fitness consequences in these systems are unclear. We provide a robust demonstration that nest-site choice covaries with offspring phenotype in *C. serpentina*, and that such variation maximizes offspring survival and, thus, maternal fitness.



Turtles, like most organisms, select oviposition sites without observing offspring performance capabilities. Thus, how could mothers “know” the abilities of their offspring so as to tailor investment for their phenotype? Such prenatal investment depending upon offspring phenotype may be possible via a positive genetic covariance between size of offspring produced and how far mothers nest from water. For example, genetic covariation is thought to maintain the association of increased offspring growth on certain host plants with maternal preference for ovipositing on those plants in *L. sativae* (Via 1986) and *E. editha* (Singer et al. 1988), as discussed above. Alternatively, variation in maternal age might drive much of the variation in investment strategy in turtles. *Chelydra serpentina* grow indeterminately throughout their lives, and larger turtles produce heavier eggs (Congdon et al. 2013; Armstrong et al. 2017; Hedrick et al. 2018). Thus, young mothers may nest closer to shore because they produce smaller offspring, but may nest farther from water as they produce larger offspring later in life. Similarly, *C. picta* produce heavier eggs and nest farther from water with age (Harms et al. 2005). However, increasing predation risk to adult females the farther they travel from water could drive such age-specific variation in nest-site choice (Harms et al. 2005; Paitz et al. 2007; but see Refsnider et al. 2015). Younger mothers may nest closer to shore to maximize their own survival, whereas older females may tolerate greater risk to themselves because they have fewer future reproductive opportunities (i.e., terminal investment hypothesis, Williams 1966) or may have lower risk because of their larger body size (Tucker et al. 1999). Optimal maternal investment in aquatic turtles, as perhaps in all oviparous taxa, may then be shaped by a complex interaction of risk to the mother, risk to the nest, and offspring dispersal ability.

Our findings suggest offspring phenotype influences nesting strategy. However, interpretation of causation is limited because our test of the offspring phenotype-nest site

covariation was correlative and not experimental. Thus, an alternative explanation could be that mothers invest in egg size (and thus indirectly in offspring dispersal ability) depending on how far they will nest from water (i.e., anticipatory maternal effect; Marshall and Uller 2007; Kotrschal et al. 2012). However, this interpretation is unlikely because egg size increases with maternal size (as discussed above) and offspring benefit from larger egg size in more aspects than just enhanced dispersal ability (increased hatching success, reduced predation risk during early aquatic life, improved competitive ability, etc.; Froese and Burghardt 1974; Janzen and Warner 2009). Thus, as they age, mothers probably produce larger offspring to maximize multiple aspects of early-life success, including dispersal ability, which frees them to nest farther from shore to lower predation risk of nests.

Maternal investment tradeoffs are shaped by environmental context and intrinsic female traits. However, optimal investment may also vary by offspring phenotype. Yet few studies have assessed the role of offspring phenotype on nesting strategy and none have shown how such covariation affects maternal fitness. We provide the first evidence for the assumption that increasing dispersal distance from nests to post-natal habitat elevates offspring mortality in animal taxa. This effect was also size-dependent, such that smaller offspring were more severely affected by increasing dispersal distances. Moreover, females that produced larger and better dispersing offspring constructed nests farther from water than females that produced smaller and poorer dispersing offspring. Thus, females that produced better dispersing offspring maximized the benefits of lower nest predation farther from water, whereas females producing poorer dispersing offspring presumably were constrained to nest closer to water so their offspring would have a reasonable chance of dispersing successfully if they hatched. Collectively, these findings elucidate the sensitivity of female investment in offspring phenotype and identify that

covariation between nest-site choice and offspring phenotype maximizes offspring survival and maternal fitness. Further considering the role of offspring performance on maternal behavior will shed light on this underexplored influence of pre-natal investment strategies.

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Table 1. Statistical results of models of survival and dispersal of hatchling *Chelydra serpentina*.

\* denote independent terms that were not significant and were removed from the final models.

For models with the dependent variable ‘nest distance to water’, all independent terms were regressed separately because independent variables were correlated with each other.

Dependent variable	Independent variable	r	df	Test Statistic	P
Survival	Mass		1, 409	$F = 1.46$	0.2276
	Release Distance		2, 409	$F = 44.69$	<0.0001
	Replicate		1, 409	$F = 5.56$	0.0189
	Mass x Release Distance*		2, 407	$F = 1.80$	0.1662
Time to Disperse	Mass		1, 206	$F = 3.49$	0.0632
	Release Distance		2, 206	$F = 30.81$	<0.0001
	Replicate		1, 206	$F = 1.66$	0.1991
	Mass x Release Distance*		2, 204	$F = 1.48$	0.2291
Dispersion Along Fence	Mass		1, 206	$F = 0.28$	0.5977
	Release Distance		2, 206	$F = 32.51$	<0.0001
	Replicate		1, 206	$F = 7.62$	0.0063
	Mass x Release Distance*		2, 204	$F = 0.01$	0.9940
Nest Distance to Water	Average Offspring Mass	0.6023	13	$t = 2.61$	0.0113
	Offspring Survival at 25m	0.0100	13	$t = 0.04$	0.4845
	Offspring Survival at 62.5m	0.0900	13	$t = -0.31$	0.5000
	Offspring Survival at 100m	0.4806	13	$t = 1.90$	0.0410

Table 2. Standardized average selection gradients quantifying the linear ( $\beta$ ) and quadratic ( $\gamma$ ) effects of straight carapace length (SCL) and mass on the survival of hatchling *Chelydra serpentina* during experimental dispersal. Significant effects are bolded.

Release Distance	<i>n</i>	Variable	Standard Deviation		Average Selection Gradient	SE	Chi-square	<i>P</i>
25	145	SCL	1.62	$\beta$	-0.0134	0.0305	0.1933	0.6602
25	145	SCL	1.62	$\gamma$	0.0288	0.7436	0.0015	0.9691
25	145	Mass	1.35	$\beta$	-0.0023	0.0313	0.0054	0.9413
25	145	Mass	1.35	$\gamma$	0.2457	0.2894	0.7212	0.3957
62.5	142	SCL	1.51	$\beta$	0.0519	0.1019	0.2590	0.6108
62.5	142	SCL	1.51	$\gamma$	0.8640	2.6892	0.1032	0.7480
62.5	142	Mass	1.32	$\beta$	-0.0264	0.1013	0.0679	0.7945
62.5	142	Mass	1.32	$\gamma$	0.4631	1.0993	0.1775	0.6735
100	141	SCL	1.61	$\beta$	0.4299	0.1656	6.7357	<b>0.0095</b>
100	141	SCL	1.61	$\gamma$	-5.4800	6.5992	0.6896	0.4063
100	141	Mass	1.35	$\beta$	0.3187	0.1489	4.5826	<b>0.0323</b>
100	141	Mass	1.35	$\gamma$	-3.2915	2.1866	2.2659	0.1322

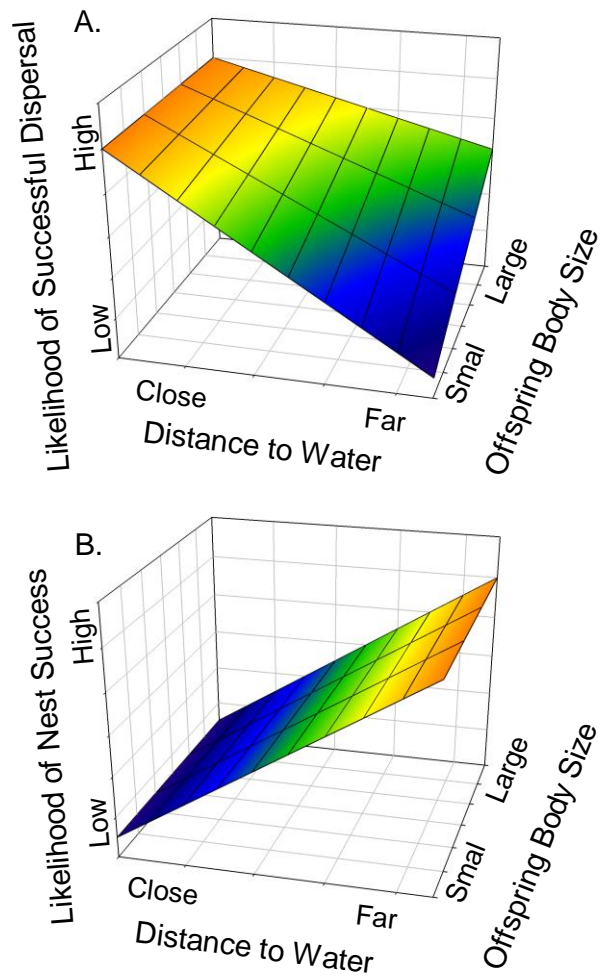


Figure 1. (A) Predicted dispersal success of hatchling turtles as a function of the interaction between hatchling body size and how far nests are laid from water. (B) Generalization of nest success (i.e., not depredated) increasing with distance to water. We predict optimal nest-site placement is a tradeoff between nest success increasing farther from water with offspring dispersal success decreasing farther from water. However, we also predict larger offspring will be less affected by increasing dispersal distances compared to smaller offspring. Thus, optimal distance from water for turtle nests should reside where the surfaces of panels A and B intersect. Panel A is a hypothetical surface based on our predictions, whereas panel B is a generalized

surface based on previous work (Kolbe and Janzen 2002a; Marchand and Litvaitis 2004; Strickland et al. 2010).

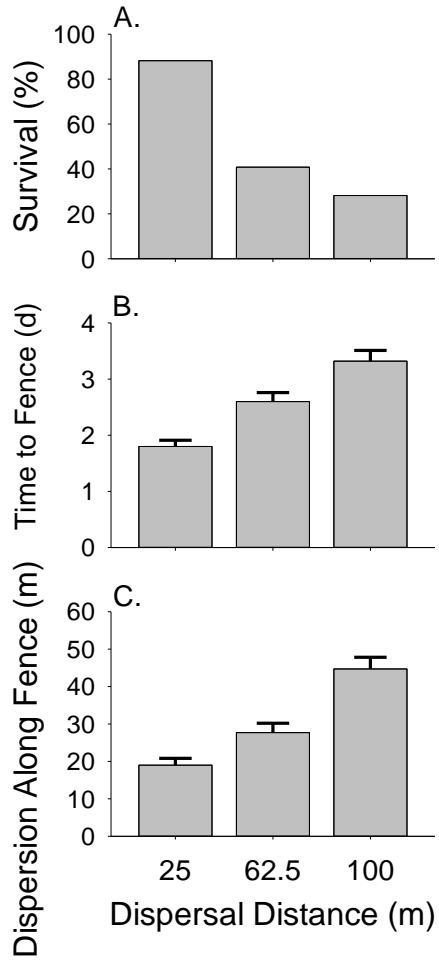


Figure 2. Effects of dispersal distance on A) survival, B) time to fence, and C) dispersion along the fence of hatchling *Chelydra serpentina* during experimental dispersal. Data are plotted as least squares means with standard errors. Statistical results are reported in Table 1.

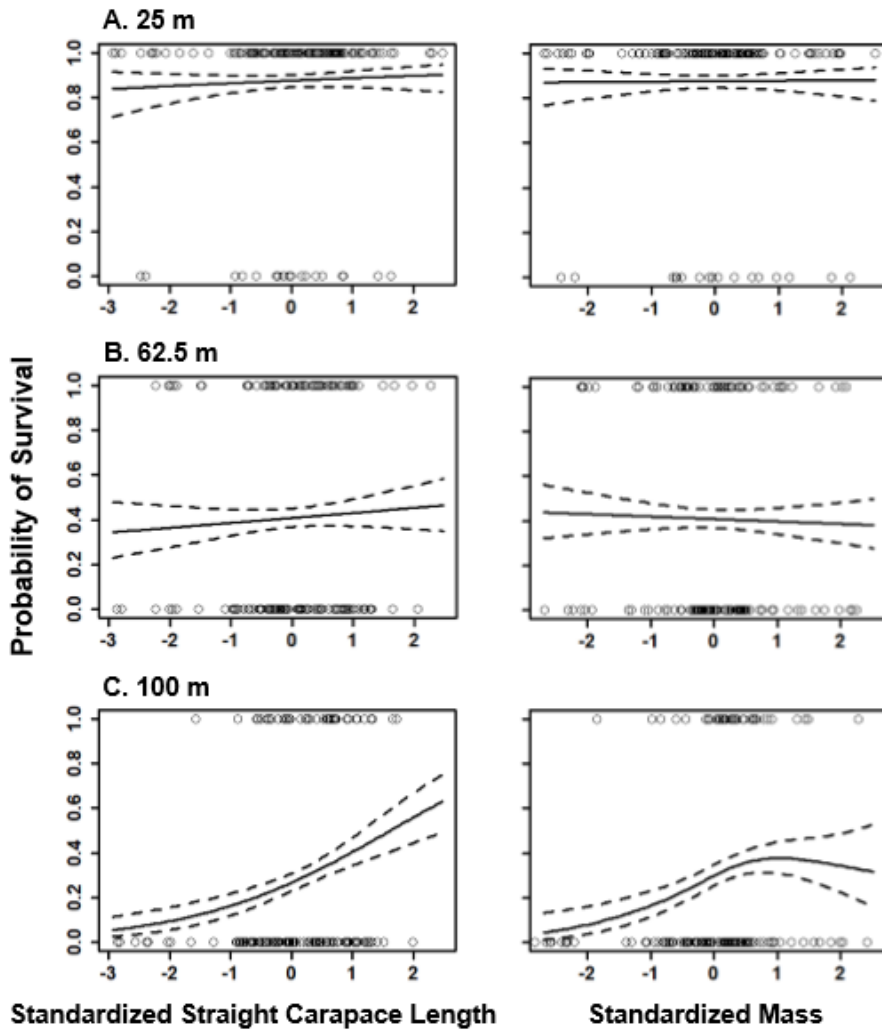


Figure 3. Effects of standardized straight carapace length and mass on the probability of survival for hatchling *Chelydra serpentina* as they dispersed from “nests” (A) 25 m, (B) 62.5 m, and (C) 100 m to a drift fence. Probability of survival was estimated using cubic splines, and dashed lines represent standard errors (Schluter 1988). Open circles along the top and bottom axes represent individual hatchlings. Statistical results are reported in Table 2.

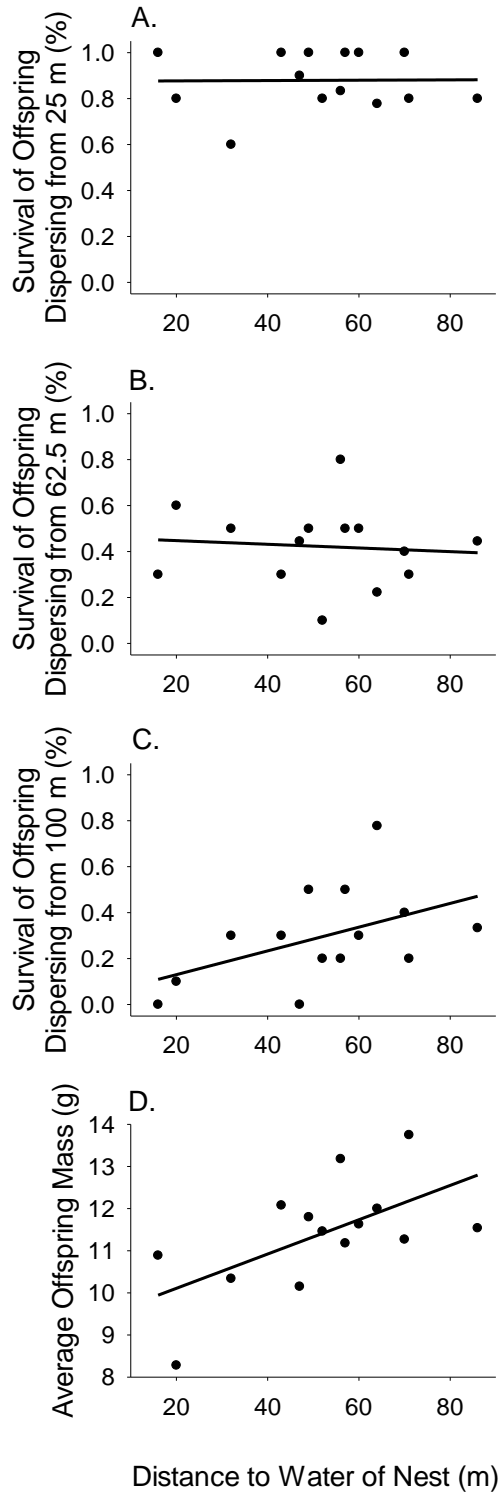


Figure 4. Relationships between the distance female *Chelydra serpentina* oviposited from water and the survival of offspring dispersing from A) 25 m, B) 62.5 m, and C) 100 m, and D) average offspring mass. Statistical results reported in Table 1.



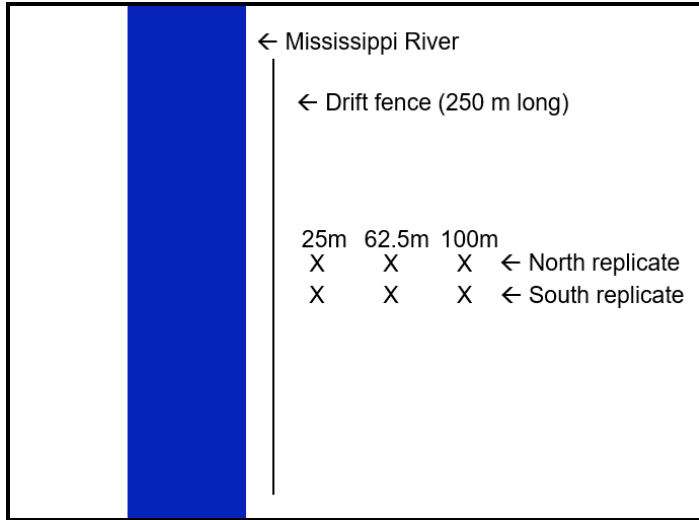


Figure S1. Schematic of experimental drift fence design. Xs denote locations where hatchling *Chelydra serpentina* were released.

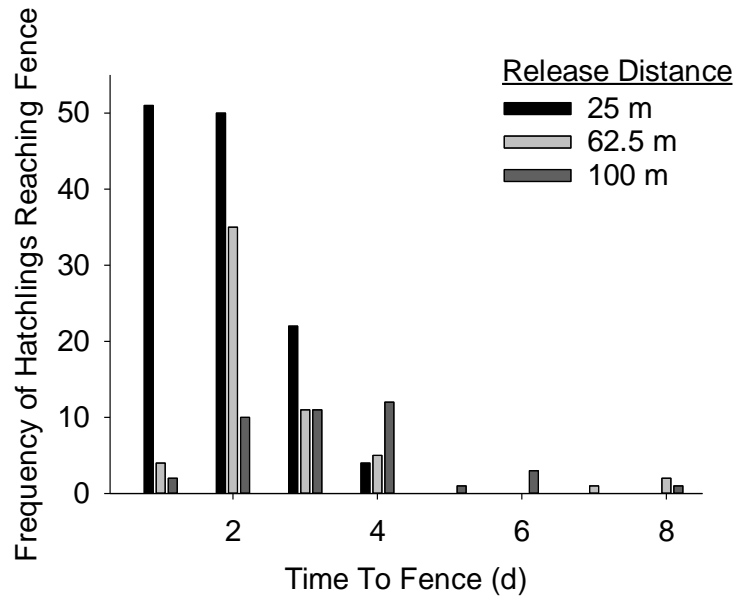


Figure S2. Effect of release distance on the time required for hatchling *Chelydra serpentina* to reach the fence during experimental dispersal. Statistical results are reported in Table 1.

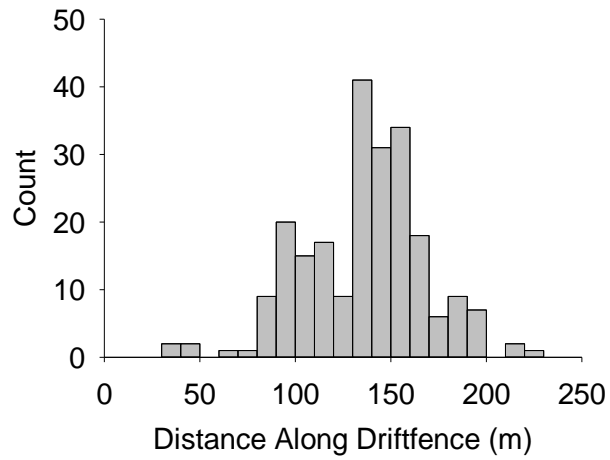


Figure S3. Distribution of locations that hatchling *Chelydra serpentina* were recaptured along a 250 m drift fence during experimental dispersal. Pitfall traps were spaced every 5 m along the fence and bin width is 10 m.

## CHAPTER 4. AGE PREDICTS RISKY INVESTMENT BETTER THAN RESIDUAL REPRODUCTIVE VALUE

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### Abstract

Life-history theory predicts that investment into reproduction should increase as future reproductive opportunities (i.e., residual reproductive value, RRV) decrease. Researchers have thus intuitively used age as a proxy for RRV and assume RRV decreases with age when interpreting age-specific investment. Yet, age is an imperfect proxy for RRV and may even be a poor correlate in some systems. We used a 30-year study of the nesting ecology of painted turtles (*Chrysemys picta*) to assess how age and RRV compare in explaining variation in a risky investment behavior. We predicted that RRV would be a better predictor of risky investment than age because RRV accounts for variation in future reproductive potential across life. We found that RRV increased after initial reproduction for a few years, slowly decreased until midlife, and then steadily decreased to terminal reproduction. However, age predicted risky behavior better than RRV. This finding suggests stronger correlates of age (e.g., size) may be

more responsible for this behavior in turtles. This study highlights that researchers should not assume that age-specific investment is driven by RRV and that future work should quantify RRV to more directly test this key element of life-history theory.

## **Introduction**

Parents invest resources into offspring to increase fitness. For iteroparous species, parents balance the benefits of investing in reproductive bouts with the costs to themselves and future reproduction (Kirkwood 1977; Roff 1992; Stearns 1992). Specifically, much theoretical work posits parents should invest more heavily into reproduction as the likelihood of future reproductive opportunities decreases (i.e., terminal investment hypothesis, Williams 1966; Gadgil and Bossert 1970; Pianka and Parker 1975; Clutton-Brock 1984). Indeed, many animal taxa increase reproductive output as they age, including insects (Creighton et al. 2009; Heinze and Schrempf 2012), amphibians (Brannelly, et al. 2016), reptiles (Massott et al. 2011; Warner et al. 2016), birds (Hanssen 2006; Velando et al. 2006), and mammals (Weil et al. 2006; Hoffman et al. 2010). Additionally, immune challenge increases immediate reproductive effort in birds (Bonneaud et al. 2004; Hanssen 2006; Velando et al. 2006; Bowers et al. 2012) and rodents (Weil et al. 2006), presumably because of a diminished likelihood of future reproduction. The occurrence of such tradeoffs is a key foundation of life-history theory.

Residual reproductive value (RRV) is the amount of reproductive potential remaining in an individual's life (Williams 1966; Pianka and Parker 1975). Females with higher RRV are predicted to invest more into themselves and future reproduction, whereas females with lower RRV are expected to invest more heavily into remaining reproductive bouts. Researchers have thus intuitively used age as a proxy for RRV (e.g., Cameron et al. 2000; Heinze and Schrempf 2012; Takata et al. 2016; Delaney and Janzen 2020) and often imply age and RRV are inverses

of each other. Yet reproductive output and survival, which determine RRV, often vary nonlinearly across age. For example, younger females may invest less into clutch size or offspring mass because of morphological and/or physiological constraints (Tucker et al. 1978; Bowden et al. 2004), whereas older females may decline in their physical ability to invest in offspring (Rockwell et al. 1993; Warner et al. 2016). In such systems, age is an imperfect correlate of RRV. Thus, more direct estimation of RRV should provide better assessment of life-history theory than the traditional proxy of age.

Despite its important role, RRV is rarely used, likely because of the difficulty in acquiring sufficient data. The formulae proposed by Williams (1966) and Pianka and Parker (1975) require age-specific survival and fecundity estimates at the population level. To calculate RRV for a given age, the products of survival to each subsequent age multiplied by the average fecundity at that age are summed. In the systems thus far studied, RRV is low in early life, because of low reproductive output and/or low survival, increasing during reproductively prime years, and then declining to late life (Pianka and Parker 1975; Vahl 1981; Bayne et al. 1983; Thompson 1984). More recently, researchers have begun redefining RRV at the individual level (Fisher et al. 2018; Moschilla et al. 2018). Here, reproductive output is measured for each individual over their entire lives and RRV is calculated as the amount of reproduction remaining in their life at given timesteps. However, such individual-level measurement of RRV across life is unfeasible for most systems, especially in the field. More importantly, this method does not account for mortality risk to subsequent ages, but instead accounts for actual mortality, which is unknown by the individual at the time of investment. Thus, despite early work, little effort has been made to test life-history theory with empirically-derived RRV.

To empirically assess this key foundation of life-history theory, we examined a long-term dataset of painted turtle (*Chrysemys picta*) investment to test whether metrics of RRV better predict investment behavior than the common proxy of age alone. We calculated three metrics of age-specific RRV (sensu Williams 1966; Pianka and Parker 1975): 1) residual clutch quantity, 2) residual egg quantity, and 3) residual clutch mass. Each of these ‘residual’ terms represent the expected amount of each reproductive output for an individual of a given age. We then compared them amongst each other and with age to test which better predict how far female *C. picta* construct nests from water. Nest distance to water is an important investment measure because maternal risk should increase the farther females travel from the safety of water (discussed in Delaney et al. 2017; Delaney and Janzen 2020). In contrast, embryos in nests constructed farther from water experience lower predation rates because nest predators tend to forage along the shore (e.g., Kolbe and Janzen 2002; Spencer 2002; Strickland et al. 2010). Moreover, females tolerate greater risk and nest farther from water as they age, which has been interpreted as being driven by reduced RRV (Harms et al. 2005; Paitz et al. 2007; Refsnider et al. 2015; Delaney and Janzen 2019, 2020). Thus, we predicted RRV would explain more variation in how far females nested from water than age alone because these metrics would account for any discordance in future reproductive potential across age.

## Methods

We leveraged data from a long-term study on the nesting ecology of *C. picta* from northwest Illinois along the Mississippi River (Warner et al. 2010; Refsnider and Janzen 2016; Warner et al. 2016). We monitored a nesting area at the Thomson Causeway Recreation Area during May and June from 1988–2018 and recorded nearly all nesting events. We located

females visually by transecting the area hourly from 0500–2000 h and allowed females to nest undisturbed. After nesting ( $n = 1,988$  nests), we captured each female, recorded shell morphometrics, estimated age based on pectoral scute growth annuli and prior observations, and notched marginal scutes to uniquely identify individuals during future nesting events (Hoekstra et al. 2018). We then excavated nests, noted clutch size, and weighed each egg. We placed eggs back into the cavities and reconstructed the nests. We also measured how far a subset of nests ( $n=1,210$ ) was constructed from water using Cartesian coordinates and the programs INTERPNT (Boose et al. 1998; as in Kolbe and Janzen 2002) and ArcView (ESRI Inc. 1998) from 1988–2010 and with a laser rangefinder thereafter (Nikon Aculon).

### *Analysis*

We performed analyses in R (version 3.6.0). We conducted all mixed-effect models using the package lme4 (Bates et al. 2015) and included female identity as a random effect to control for the multiple observations of most females. We restricted our analysis to only ages with  $\geq 10$  observed nests to increase our confidence in age-specific parameter estimates.

We first plotted reproductive investment across age and visualized the fit of the data with locally estimated scatterplot smoothing (LOESS) regression lines (Jacoby 2000). Based on these figures, we ran a series of general linear mixed models to assess the linear and quadratic relationships between age and reproductive metrics. We used clutch size, mean egg mass, number of clutches laid per year (clutch quantity), total number of eggs laid per year (egg quantity), and total clutch mass per year (clutch mass) as dependent variables. We used age and  $\text{age}^2$  as independent variables.



We calculated RRV for each age following methodology in Williams (1966) and Pianka and Parker (1975) using the following equation:

$$\sum_{t=x+1}^{\omega} \frac{l_t}{l_x} m_t$$

Subscripts  $x$  and  $t$  represent age, and  $\omega$  is the last age of reproduction. Term  $l$  represents survivorship and  $m$  represents fecundity. We used clutch quantity, egg quantity, and clutch mass as measures of fecundity and thus calculated three metrics of RRV. We estimated age-specific survival rates using the package RMark (Laake 2013) and allowed detectability to vary by time (most supported age-specific survival model). Age-specific survival rates were generally high (>80%, Supplementary Fig. 1).

To compare RRV and age in predicting investment behavior, we first assessed distance to water across age and RRV with LOESS regression. We then ran mixed-effect regressions with distance to water as the dependent variable. We used either age or one of three RRV metrics as the independent variable in four separate models. We compared the fit of each model with AIC values and the amount of variation explained (Burnham and Anderson 1998). Based on these models, we also ran a regression with plastron length as the independent variable.

## Results

We analyzed data from 1,988 nesting events of known-age females of which distance to water data existed for 1,210 nests. The distribution of known-age females was skewed with more observations of younger females (Fig. 1F). Still, we observed females  $\geq 10$  times for each age from 5–22 years old, and focused our analysis on this age range.

Although age had a quadratic effect on clutch size, females produced ~10 eggs on average per reproductive bout across life (Fig. 1A). On the other hand, females produced larger

eggs and more clutches annually with age (Fig. 1B & C; Table 1). Females thus also produced more eggs per year and heavier total clutch mass per year as they aged (Fig. 1D & E; Table 1). In addition, mean egg mass, number of clutches laid per year, eggs laid per year, and clutch mass produced per year all had significant quadratic terms such that the increase in investment plateaued to varying degrees (Fig. 1B–E; Table 1).

Residual reproductive values increased initially from age 5 to 6 and 7, then slowly decreased until mid-life, and then steadily decreased (Fig. 2). Regardless of this general pattern, age explained more variation in distance to water than any RRV metric (Fig. 3. Table 2). Specifically, age explained 7% of the variation in distance to water compared to 4–5 % for RRV metrics. Plastron length of females explained 8% of the variation in distance to water ( $F_{1, 1151} = 70.11, P < 0.0001$ ). The random effect of female identity accounted for another 27–28% of variation depending on the model (Table 2).

## Discussion

Life-history theory predicts iteroparous species should invest more into reproduction as RRV decreases. Age has served as an intuitive proxy for RRV to assess this theory, yet in principle the quantification and use of RRV in models should be more appropriate. We leveraged a 31-year dataset on the nesting ecology of *Chrysemys picta* to test if RRV explains investment behavior better than age. We found that reproductive output and RRV varied nonlinearly across female age, which highlights that age is not a perfect proxy for RRV. However, age explained more variation in how far turtles nested from water than any metric of RRV. Perhaps another correlate of age, such as size-dependent predation risk, contributes to how far turtles nest from

water more than previously appreciated. Regardless, our results suggest researchers should not simply interpret age-related changes in investment as being driven by RRV.

In our system, RRV increased in early life, maintained high but decreasing levels through midlife, and steadily decreased in late life. This trend was driven by reproductive output generally increasing with age early in life and then plateauing or declining to late life (Warner et al. 2016) and imperfect survival to subsequent ages (Warner et al. 2016; Reinke et al. 2020). Thus, RRV is not the inverse of age in this system. In fact, the shape of RRV across age in our population matches other wild populations for which RRV has been derived, including three lizards and a snake (Pianka and Parker 1975), a scallop (Vahl 1981), and a mussel (Bayne et al. 1983; Thompson 1984; see also Descamps et al. 2007 for similar reproductive value). Moreover, because RRV and age differ from each other, we should expect behaviors that are driven by future reproductive potential to more closely correlate with RRV than age.

Despite such expectations, age predicted the distance females nested from water better than any metric of RRV. Thus, a different trait that correlates with age may be a greater driver of how far females construct nests from water. For example, body size increases nearly linearly with female age after about 7 years old (see Fig. 1 in Hoekstra et al. 2018), and smaller turtles may be more susceptible to predation during terrestrial nesting forays (Tucker et al. 1999). In support of this possibility, female body size accounted for a similar amount of variation in how far females nested from water as age. Therefore, females may venture farther from the safety of water as they age because their larger size lowers predation risk. Such size-biased predation risk could be the result of more robust shell morphology or increased locomotor ability with size, although preliminary evidence suggests locomotor ability does not vary with adult female size or age (Delaney unpublished). Also, females produce larger offspring as they grow (Iverson and

Smith 1993; Rowe et al. 2003), and bigger offspring typically have better survival during dispersal (Janzen 1993; Tucker 2000; Janzen et al. 2000a, 2000b; Janzen et al. 2007; Paitz et al. 2007) and are capable of longer dispersal distances than smaller offspring (Delaney and Janzen 2019). Thus, females may nest farther from water with age because their larger offspring are better able to handle longer dispersal to water compared to the smaller offspring from younger mothers. Another possibility is that remaining lifespan, excluding mortality risk, drives risk-taking behavior. This would be the inverse and linear correlate of age. However, investment based on such a metric should not maximize lifetime fitness as optimally as RRV.

Previous empirical tests of the theory that reproductive effort should increase as RRV decreases have not supported this idea. Reproductive effort (i.e., output divided by female mass) tends to increase with age to varying degrees (Pianka and Parker 1975; Vahl 1981; Bayne et al. 1983; Thompson 1984; Dobson and Jouventin 2010). In these studies, RRV also increased with age to some point after which RRV decreased until the last age of reproduction. Thus, tradeoffs between reproductive effort and RRV may only have existed at the end of reproductive life (i.e., terminal investment, Williams 1966). Similarly, we show that reproductive effort in terms of nest distance to water increased with age and that RRV is quadratic across age. Research that defines RRV as the amount of reproduction remaining in life at the individual level have also found that age is a better predictor of risk-taking behavior than their metrics of RRV (Fisher et al. 2018; Moschilla et al. 2018). How to compare findings between population-specific and individual-specific RRV is unclear though because the latter accounts for actual mortality instead of the probability of survival to subsequent ages, which should produce RRV measures that correlate strongly with age. Individuals should behave according to the likelihood of future reproduction

rather than actual future reproduction because actual future reproduction is not known at the time of investment (except perhaps in rare cases of terminal investment).

Age and RRV were highly significant predictors of the distance females nested from water, yet explained just 7% and 4–5% of the total variation, respectively. Maternal identity accounted for an additional 27–28% of variation in distance to water, which suggests female preference for nest distance to water is repeatable and likely heritable (Delaney et al. in review; Janzen et al. 2019). Despite the low variation explained by age alone, age plus female identity accounted for 34% of the variation. Nest-site choice is a complex behavior and oviparous animals must satisfy a variety of requirements to maximize offspring development and success (Resetarits 1996; Refsnider and Janzen 2010; Moore et al. 2019). For example, females must select sites with appropriate thermal and hydric conditions, which are influenced by nest substrate (Hays et al. 2001; Tornabene et al. 2018; Mitchell and Janzen 2019) and overstory canopy cover (Janzen 1994; Weisrock and Janzen 1999; Mitchell et al. 2013; Refsnider et al. 2013; Pruett et al. 2019). Females may also encounter extrinsic stressors that influence nest-site choice, such as terrestrial predators (e.g., raccoons) or human activity (e.g., Spencer 2002). Anthropogenic disturbance is not uncommon at our site as turtles are nesting in an area of high human activity (i.e., a campground). However, females do not appear to alter nest distance to water following rare handling by humans (Delaney and Janzen 2020) nor does the presence of recreationists alter the number of turtles that emerge from water to nest (Bowen and Janzen 2008). Despite the number of factors that shape nest-site choice, age and RRV (to a lesser degree) explained appreciable variation in how far females nested from the safety of water.

A few statistical points bear important ramifications for this project. First, we tested how age and RRV explain linear variation in how far females nested from water. However, quadratic

variation in RRV could drive the same biological response in distance to water as age does linearly. To restate this another way, the same response in Z could be driven linearly by X or quadratically by Y, by different mechanistic patterns. If RRV influences variation in nest distance to water, our findings suggest RRV perhaps does so in a nonlinear fashion (Fig. 3). Second, we first estimated metrics of reproductive output to calculate RRV and then used the calculated RRV to predict distance to water. However, our estimates of reproductive output contained some level of uncertainty at each age. If the error estimates are heteroscedastic across age, then the slope of Y on X will be biased. We combatted this in our study by only focusing on ages for which we had large enough sample sizes to keep estimate errors low. However, errors increased for estimates of reproductive output for the terminal few ages in our study (Fig. 1 and Supplementary Fig. 1). The accompanying decrease in slope of RRV on distance to water during the terminal few ages (Fig. 3) may be a product of this statistical bias. These statistical issues will be important to consider for future work on RRV.

We show that age explained more variation in a risky investment behavior than RRV in a turtle. Our results highlight that researchers should not assume age-related changes in investment are driven by RRV. Future work should more directly assess life-history theory by quantifying RRV and examining its relationship with reproductive effort. Systems with strongly nonlinear reproductive output or nonlinear risk-taking or reproductive behaviors (e.g., parental care) across life may be especially well suited for empirical assessment. In fact, long-term studies of reproductive investment may already have data to estimate age-specific RRV (e.g., Festa-Bianchet and King 2007; Sparkman et al. 2007; Hayward et al. 2013; Murgatroyd et al. 2018).

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Table 1. Linear ( $\beta$ ) and quadratic ( $\gamma$ ) effects of female age on metrics of reproductive investment in *Chrysemys picta*.

Dependent Variable	Effect of Age	F	P
Clutch Size	$\beta$	$F_{1, 1986}=1.7$	0.1984
	$\gamma$	$F_{1, 1986}=11.4$	0.0008
Mean Egg Mass	$\beta$	$F_{1, 1986}=1438.0$	<0.0001
	$\gamma$	$F_{1, 1986}=239.1$	<0.0001
Clutches Laid per Year	$\beta$	$F_{1, 1433}=14.8$	0.0001
	$\gamma$	$F_{1, 1433}=18.4$	<0.0001
Eggs Laid per Year	$\beta$	$F_{1, 1433}=14.6$	0.0001
	$\gamma$	$F_{1, 1433}=23.7$	<0.0001
Clutch Mass per Year	$\beta$	$F_{1, 1433}=157.5$	<0.0001
	$\gamma$	$F_{1, 1433}=67.5$	<0.0001
Distance to Water	$\beta$	$F_{1, 1118}=76.4$	<0.0001
	$\gamma$	$F_{1, 1118}=2.1$	0.1512

Table 2. Comparisons between metrics of residual reproductive value (RRV) and reproductive age (years) on the ability to explain variation in how far female *Chrysemys picta* nested from water. Independent variables are listed from highest to lowest order of variation explained.  $R^2_m$  represents the variation explained by the fixed effect;  $R^2_c$  represents the variance explained by the fixed effect plus the random effect of female identity.

Independent Variable	Delta AIC	$R^2_m$	$R^2_c$	df	F	P
Age	0.0	0.07	0.34	1, 1209	82.3	<0.0001
RRV (Clutch Quantity)	20.5	0.05	0.33	1, 1209	58.9	<0.0001
RRV (Offspring Quantity)	23.9	0.05	0.33	1, 1209	60.2	<0.0001
RRV (Offspring Mass)	39.9	0.04	0.32	1, 1209	47.4	<0.0001

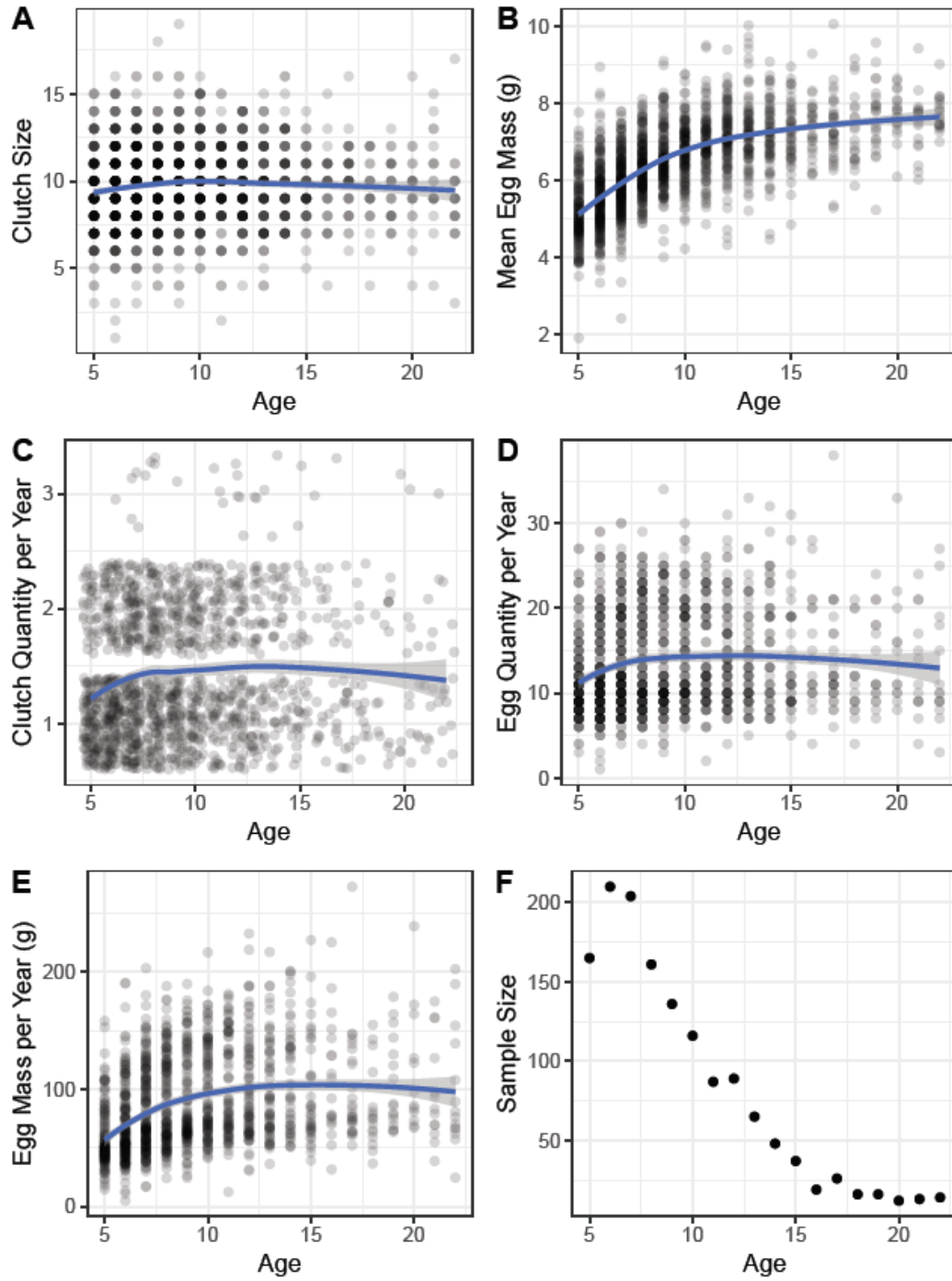


Figure 1. Measures of reproductive output (A-E) and sample size (F) across age (years) of maternal *Chrysemys picta*. Data in (C) are jittered to reduce overplotting. Lines are plotted as LOESS regressions  $\pm$  1 SE. Statistical results are reported in Table 1.

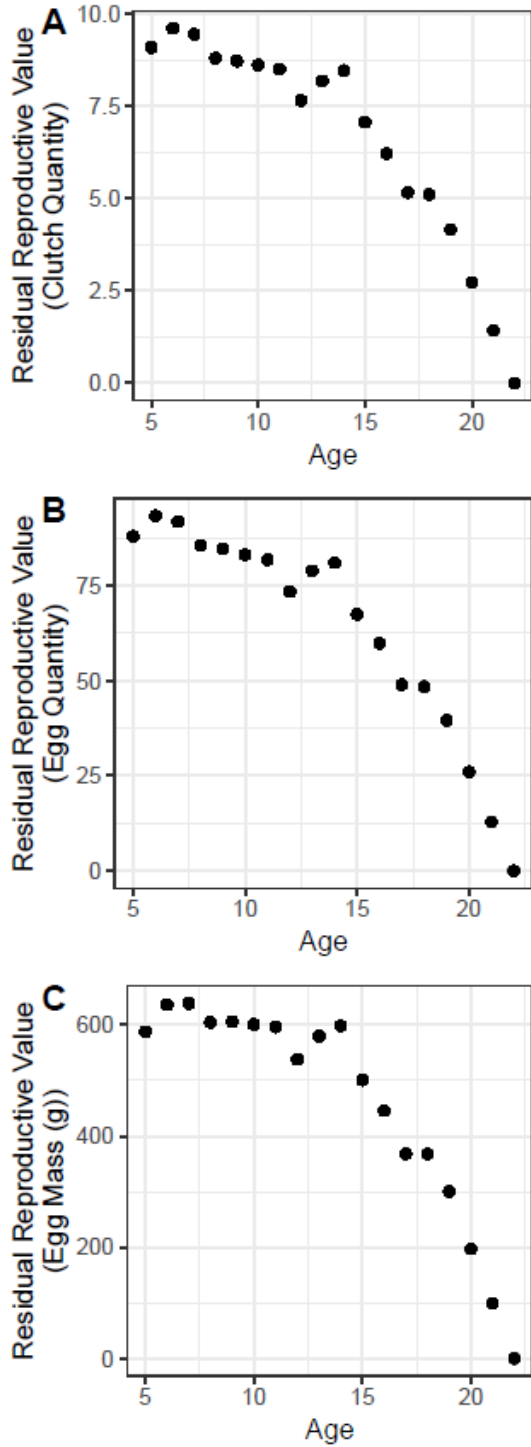


Figure 2. Correlations between reproductive age (years) of adult female *Chrysemys picta* and metrics of residual reproductive value.



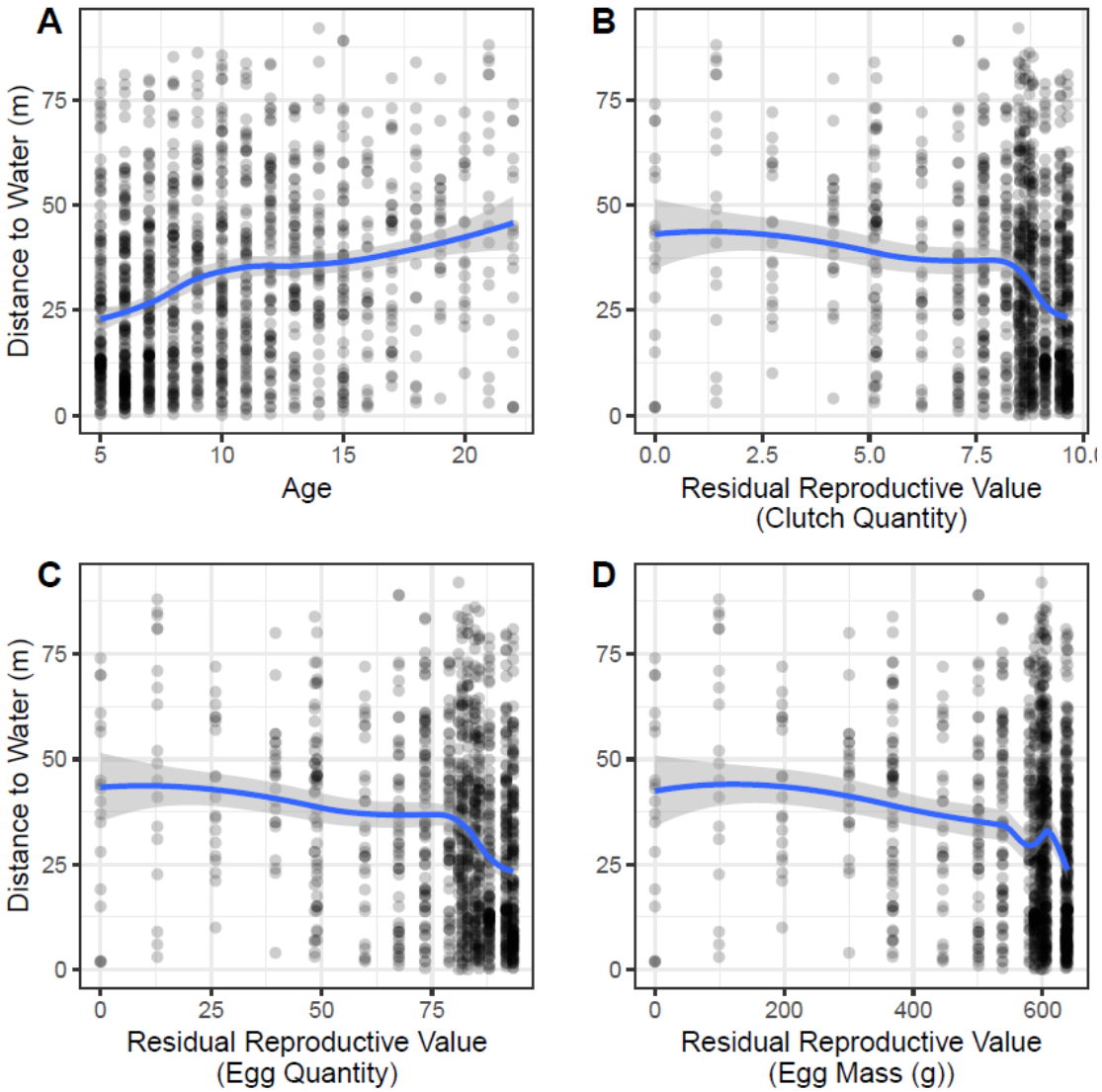
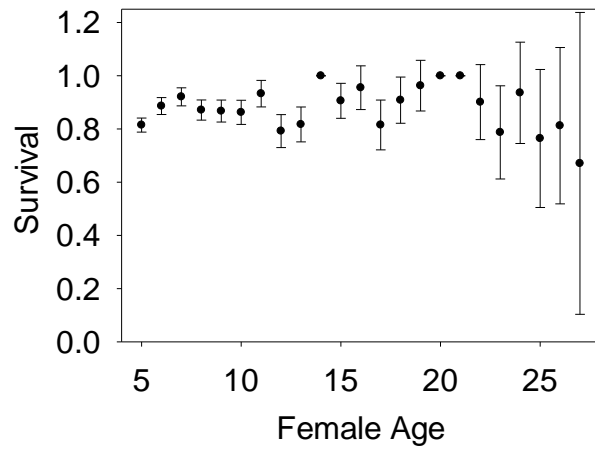


Figure 3. Comparisons between reproductive age (years) and metrics of residual reproductive value on the ability to explain variation in how far *Chrysemys picta* nested from water (see Table 2). Lines are plotted as LOESS regressions  $\pm 1$  SE.



Supplementary Figure 1. Age-specific survival estimates of adult female *Chrysemys picta*. Data are plotted as estimates  $\pm$  1 SE.

## CHAPTER 5. A SYNTHESIS

Theory predicts organisms should increase reproductive effort as residual reproductive value (RRV) decreases (Williams 1966; Pianka and Parker 1975). This theory has served as the foundation for diverse studies of age-specific reproductive strategies. Despite widespread investigation, key questions remain. Does plastic adjustment of reproductive investment depend upon the age of the organism? How does offspring phenotype affect parental reproductive investment, and does any such covariation shift across reproductive life? How does the use of age as a proxy for RRV affect interpretation of age-specific investment?

In chapter two, I tested how elevated predation risk to mothers influences nest-site choice, and subsequently how maternal response to increased risk affects offspring survival in painted turtles, *Chrysemys picta*. Furthermore, I compared young and old mothers to assess if response to risk varies depending upon age. I predicted that young mothers would invest heavily in themselves (i.e., nest closer to the safety of water) and thus future reproduction, whereas older mothers would invest more into current reproduction (i.e., nest farther from the shore because of lower nest predation risk). I found that although older females nested farther from shore than younger females, neither young nor old turtles altered the distance they nested from water after perceiving elevated risk. However, nest sites chosen after simulated predation experienced a 17% greater predation rate. This result suggests female preference for distance to water is robust to maternal risk, but females likely altered nest-site choice in a way we did not quantify that cost offspring survival. For example, females may have selected sites with different substrate composition or understory vegetation structure. Fruitful follow-up work should address this question in less human-disturbed sites, which would reduce the possibility that turtles do not

perceive handling by humans as a threat (not likely) or that turtles are always nesting under the perception of high risk (more likely).

This question is addressable in any iteroparous species for which a conflict between investment into reproduction versus maternal survival exists. For example, birds increase reproductive effort (RE) when immune challenged (Bonneaud et al. 2004; Hanssen 2006; Velando et al. 2006; Bowers et al. 2012) and reduce RE when predation risk is high (Harris 1980; Harfenist and Ydenberg 1995; Scheuerlein et al. 2001; Eggers et al. 2006; Scheuerlein and Gwinner 2006; Thomson et al. 2006; LaManna and Martin 2016, 2017). Thus, birds may be particularly well suited to address whether plastic investment shifts across the reproductive life. In fact, simply replicating these bird studies or reevaluating the data while testing for age-specific responses could suffice. I suspect uncertainty and/or low variation in female age has precluded such comparisons in previous work.

In chapter three, I assessed how offspring phenotype influences maternal investment strategy in common snapping turtles, *Chelydra serpentina*. As predicted, I found that larger hatchlings were better at dispersing longer distances than smaller hatchlings. Moreover, females that produced bigger and better dispersing offspring constructed nests farther from water than smaller and poorer dispersing offspring. These findings suggest that bigger (and older) mothers can oviposit eggs farther from water where nest predation risk is lower, whereas smaller (and younger) mothers are constrained to nest closer to the safety of water because their offspring must be close enough to disperse back to water if they survive incubation. More broadly, these findings show that female investment can be sensitive to offspring phenotype and that accounting for offspring performance maximizes maternal fitness.

I suspect future work on this issue will uncover similar maternal clutch-specific investment in systems where offspring phenotypes differentially benefit by various investment strategies. In particular, species with size-dependent performance and substantial variation in the size of young are poised to assess this question. This study is also the first to identify that offspring dispersal ability covaries with the distance offspring are oviposited from juvenile habitat, despite being hypothesized in oviparous taxa (Refsnider and Janzen 2010). Future work in systems where offspring are oviposited or birthed in locations away from juvenile habitat (e.g., depression-nesting fishes) may be well suited for addressing this question.

In chapter four, I examined how age and RRV compare in predicting how far female *C. picta* construct nests from water. Previous work has shown that older females nest farther from water than younger mothers and suggested this effect is driven by RRV. I predicted that RRV would explain more variation in distance to water than age because RRV accounts for any nonlinearity in future reproductive potential across age. I found that RRV had a more quadratic, rather than linear, pattern across age, increasing from the first age of reproduction for a few years and then decreasing to terminal reproduction. Yet, age was a better predictor of how far females nested from water than RRV. This outcome highlights the fact that researchers should not simply interpret age-related variation in reproductive behaviors as being driven by RRV. Instead, I suggest researchers should quantify RRV and assess how it compares with age and potential correlates (e.g., body size, condition, reproductive experience) for a more complete and direct assessment of life-history theory.

Despite the conceptual formalization of reproductive value (Fisher 1930) and RRV (Williams 1966) more than 50 years ago, researchers in ecology and evolutionary biology rarely quantify these values and use them to empirically assess foundational life-history theory. Instead,

researchers more commonly use age as a proxy for RRV. Despite the intuitive and widespread acceptance that RE should tradeoff with RRV, the few empirical studies that have examined this matter only find a tradeoff at the tail end of reproductive life (Pianka and Parker 1975; Vahl 1981; Bayne et al. 1983; Thompson 1984; Dobson and Jouventin 2010). Empirical work on this topic is likely hindered by the amount of data needed to estimate age-specific survival and fecundity to calculate RRV. Yet, long-term studies of reproductive ecology likely already have the required data. In addition, perhaps a fruitful direction for future research would be to identify iteroparous species that reproduce more frequently and have shorter lifespans (e.g., many arthropods) to compare RE and RRV across lifespan.

Another possibility for failing to find support for Williams' theory is that RE may be difficult to fully quantify (Clutton-Brock 1984). The traditional metric is calculated as offspring mass divided by maternal mass. More recently studies have used risk-taking behaviors as metrics of RE (Fisher et al. 2018; Moschilla et al. 2018; chapter four). However, if the importance of mass invested or risk-sensitivity of females varies across life, then the RE calculated may not represent female 'effort'. This problem will be more difficult to tackle and may require extensive knowledge of the ecology of a system to properly measure and scale RE. The reproductive ecology of freshwater turtles is particularly well studied and may be uniquely poised to address this issue. For example, we now know how numerous factors influence embryonic development and survival (Janzen 1994a, 1994b; Spencer 2002; Kolbe and Janzen 2002; Janzen and Warner 2009; Strickland et al. 2010; Mitchell et al. 2013a, 2013b; Refsnider et al. 2013; Bodensteiner et al. 2015), hatchling survival during dispersal (Janzen 1993; Tucker 2000, Janzen et al. 2000a, 2000b; Janzen et al. 2007, Paitz et al. 2007; Delaney and Janzen 2019), maternal risk and survival (Tucker et al. 1999; Spencer 2002; Refsnider et al. 2015; Polich and Barazowski 2016;

Delaney et al. 2017; Delaney and Janzen 2020), and how females shift investment across age (Harms et al. 2005; Paitz et al. 2007; Warner et al. 2016; Delaney et al. in review). Most of this work has focused on the consequences of investment for progeny. However, to improve our metric of RE, future work will need to assess how the costs of investment (e.g., Delaney et al. 2017) scale across the lifespan.

Collectively, these chapters identify multiple drivers of nest-site choice in freshwater turtles. Turtles increase how far they nest from water with age, which is likely because young turtles nest close to the safety of shore to increase their own survival, whereas older turtles nest farther from water to reduce nest predation risk. Yet, variation in distance to water also appears to be driven by offspring dispersal ability. Although I was unable to experimentally tease apart these factors, I suggest that the future reproductive potential of mothers, predation risk of nests, and size-dependent dispersal ability of offspring all interact to shape maternal nesting behavior. Size-dependent maternal risk may also contribute to how far females nest from water (Tucker et al. 1999). Females of oviparous taxa must also find appropriate substrate and vegetation structure to ensure adequate thermal and hydric conditions for development. In reptiles with temperature-dependent sex determination, the transitional range of temperatures that produce mixed-sex clutches is often quite narrow and adds another layer of complexity to the factors shaping nest-site choice (Mitchell et al. 2013). Thus, nest-site choice is complex, and females must satisfy numerous requirements (for themselves and for progeny) for successful reproduction (Refsnider and Janzen 2010). Here I show that balancing these pressures shifts with age to maximize lifetime fitness.

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