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Consequences of maternal effects on offspring size, growth and survival in the desert tortoise

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Abstract

Maternal body size can have notable consequences on reproductive success. For example, fecundity often increases with body size. Less is known, however, about the relationship between maternal size and factors affecting offspring fitness, including size, growth and survival. Here, we examined the relationship between hatchling and maternal body size in the Mojave Desert tortoise *Gopherus agassizii*. We further examined the relationships between survival and growth after 1 year and size at hatching. We found that larger females tended to produce larger offspring; post-hatching growth and survival also correlated positively with size at hatching. Our results suggest that, in desert tortoises, maternal body size may indirectly influence offspring fitness via growth and survival for at least the first year of life. Such an advantage early in life may confer long-term benefits for individuals, especially in species thought to have high juvenile mortality or that inhabit highly variable environments.

Introduction

Differential offspring performance and survival are key mechanisms by which organisms are shaped by their environments. Maternal effects – the causal influence of maternal genotype or phenotype on offspring phenotype – are increasingly recognized for their contributions to differential offspring performance (Wolf & Wade, 2009). Studies of maternal effects are thus broadening our understanding of organismal ecology and life-history evolution (Mousseau *et al*., 2009). We now know, for example, that familial identity can affect offspring survival and thus provide an alternative means to increasing maternal fitness beyond simply producing more offspring (Steyermark & Spotila, 2001).

Nutritional provisioning that results in larger neonates, eggs or embryos typically increases offspring survival (Bagenal, 1969; Ankney, 1980; Janzen, 1993; Congdon *et al*., 1999; Janzen, Tucker & Paukstis, 2000). This increased offspring survival has been attributed to larger eggs having greater lipid stores (Ankney, 1980; Congdon & Gibbons, 1985), which provide more energy for developing embryos and hatchlings (Scott *et al*., 2007). Although previous studies have examined the effect of variation in maternal size on egg size, few have examined longer-term post-hatching fitness consequences for offspring.

Oviparous reptiles are particularly useful for studying maternal effects on offspring growth and survival. Female size in turtles, for example, is correlated with clutch size and egg size (Congdon, Gibbons & Greene, 1983; Iverson & Smith, 1993; Rowe, 1995). Female body size is thus thought to have important consequences for reproductive success in turtles (Congdon & Gibbons, 1985; Turner *et al*., 1986; Wallis, Henen & Nagy, 1999; Wilkinson & Gibbons, 2005). Egg mass is generally also positively correlated with hatchling mass in turtles (Congdon *et al*., 1983; Steyermark & Spotila, 2001; Wilkinson & Gibbons, 2005). There is little evidence, however, that maternal body size is directly linked to post-hatching offspring size. Evidence that maternal choice, such as nest timing or site selection, or other maternal characteristics, including genetics and age, affect post-hatching growth rates or offspring survival is likewise mixed (Brooks *et al*., 1991; Kolbe & Janzen, 2001). For example, egg size and hatchling size had no effect on post-hatching growth or survival in snapping turtles *Chelydra serpentina* (Brooks *et al*., 1991; Kolbe & Janzen, 2001); nest characteristics such as incubation temperature or nest location did, however, affect post-hatching growth rates and survival (Brooks *et al*., 1991; Kolbe & Janzen, 2001). There is consequently a tremendous opportunity for additional work to clarify the contributions of maternal effects to offspring performance and survival in a well-studied vertebrate system.

Female body size in the Mojave desert tortoise *Gopherus agassizii* can affect both clutch size and egg size (Turner *et al.*, 1986; Wallis *et al.*, 1999); as with many turtles, however, effects on offspring body size and post-hatching fitness remain poorly studied. On average, clutch size increases by one egg with each 19 mm increase in female carapace length (Turner *et al.*, 1986). In contrast, there is yet no evidence that maternal body size influences hatchling size or survival (Spotila *et al.*, 1994). Because Mojave desert tortoises inhabit extremely arid environments with considerable variation in precipitation from year to year that drives resource availability (Hereford, Webb & Longpre, 2004; Medica *et al.*, 2012), we may expect that maternal contributions to offspring size have a great impact on offspring growth and survival. Our goal in this study was to determine the degree to which maternal body size affects offspring size and, consequently, offspring growth and survival. Given that egg size correlates with maternal body size (Congdon *et al.*, 1983) and that hatchling size correlates with egg size (Congdon *et al.*, 1983), we predicted that larger female desert tortoises would produce larger offspring. Moreover, because larger eggs in birds and turtles have greater energy provisions (Ankney, 1980; Congdon & Gibbons, 1985), we predicted that larger hatchlings would have greater growth rates and better survival in their first year. By examining this relationship, our study could provide insights about the value of large females in species that inhabit uncertain environments.

Methods

Study site

We conducted this study from 2011–2013 in Ivanpah Valley, California, USA (35°34′N 115°28′W), in the northeastern portion of the Mojave Desert. The tortoises studied here were part of a larger study on head-starting in Mojave National Preserve. Juvenile rearing pens (9 × 9 m) were located at the Ivanpah Desert Tortoise Research Facility and all juveniles included in the present study were reared entirely within these pens. Pens excluded natural predators, including ravens, coyotes and rodents, but otherwise matched the natural environment. Pens were equipped with aquifer-fed sprinkler systems to allow supplemental watering. We provided supplemental water to juvenile pens using two treatments as part of a separate study of the effect of supplemental precipitation on hatchling growth and activity. Treatments varied in frequency of water application (see Nafus, 2014 for further details). Treatments coincided with natural rainfall patterns (see Hereford *et al.*, 2004 for a review of Mojave Desert climate and precipitation patterns) and we did not water during the dry seasons when rainfall is uncommon in the Mojave Desert (end of May–July). Thus, supplemental water was only applied seasonally. We also did not provide juveniles with supplemental forage.

Offspring and maternal body size relationship

From April 2011 to August 2012, we captured free-ranging adult female tortoises. At first capture, we notched a unique combination of marginal scutes using a triangular file to assign each female an identification number (Cagle, 1939) and affixed a radio transmitter (RI-2B, Holohil Systems Ltd., Carp, ON, Canada) to each animal's carapace. As stipulated by our permits, we attached transmitters and antennas (total attached weight < 5% of body mass) to costal scutes. At initial capture and each subsequent handling, we collected the mid-line carapace length (MCL), as measured by the distance from the nuchal to pygal scutes (±1 mm), and mass (±50 g).

We radiographed females every 10–14 days from April to July 2011 and 2012 (Diagnostic Imaging Systems, Inc., Poskum Model PXM-20BT, Rapid City, SD, USA; 60 kVp, 0.8 mAs, 74 cm focal length) to detect and count the number of calcified eggs present in each female (Gibbons & Greene, 1979). We isolated gravid females in predator-excluded pens (one tortoise per pen) for nesting, after which we returned females to the burrow from which they were collected. We also released any gravid females that had not nested after 30 days of captivity. Due to permit restrictions, we only radiographed and collected the first clutch from each female in a given year.

We attempted to find all nests in pens, but did not disturb nests to count or manipulate eggs. We searched the nesting pens daily from August to September in 2011 and 2012, beginning 80 days after the estimated nesting date. We removed hatchlings from nesting pens when they emerged from nests and immediately weighed (to nearest 0.1 g) each animal and measured their MCL (±0.1 mm). We also individually marked hatchlings by notching marginal scutes using nail clippers for later identification.

We used raw data values in our statistical models unless otherwise specified and accepted significance at $\alpha = 0.05$. We measured Pearson's product-moment correlation coefficient between MCL and mass in adult females and juveniles using the Hmisc package in program R (Harrell, 2015). Because mass and MCL were highly correlated for both demographics, we used only MCL in our comparisons. Tortoises easily gain 10% of their mass following drinking and can lose a similar percentage after a single void of their bladder (unpubl. data); mass is therefore a less reliable measure of size than carapace measurements.

We used a linear mixed-effect model [lme4 package in R (Bates *et al.*, 2013)] to determine whether offspring MCL correlated with maternal MCL. We calculated the mean clutch MCL for each female and set them as the dependent variables. We included maternal MCL and clutch size as independent fixed effects. As clutch size has been correlated with maternal MCL in this species (Turner *et al.*, 1986), we included an interactive effect between them in our model. Because a subset of the same females contributed offspring in 2011 and 2012, we included female identification number and year as random effects in our models.

Post-hatching offspring survivorship

After all hatchlings emerged each year, we assigned them to rearing pens using a stratified block design to ensure that no more than one hatchling from a given mother resided in a pen. Hatchlings densities were approximately one tortoise per

10 m^2. We documented any mortalities of captive juvenile tortoises for the 12 months following hatching. Additionally, during the months of February–May, and again in September, we searched each pen multiple times per day, recording every individual encountered. Searches were limited to detecting animals active on the surface or seen within the first 0.5 m of burrows. We treated any individual that was never seen during these intensive pen searches as having died.

We used a logistic mixed-effect model [lme4 package in R (Bates *et al.*, 2013)] to measure the effect of MCL at hatching on survival. In our survival model, the dependent variable of first-year survival was binomial; animals either died (0) or survived (1). We included MCL at hatching as a fixed independent effect. Because we included multiple individuals from the same clutch, maternal identification number was included as a random effect in order to appropriately partition within clutch variability. Additionally, we included cohort year as a random effect because of the large differences in post-hatching survival between years. Precipitation treatment was not included in our model because of equal mortalities occurring in both treatments and to reduce parameter load.

Post-hatching offspring growth

For the 2011 cohort, we measured MCL 1 year post-hatching (*n* = 39) to calculate growth rate (mm per day) during the first year. Using the MCL at hatching (MCL_i) and the final measurements collected in September 2012 (MCL_f), we calculated daily growth for each individual, where *d* was the difference in days between the first capture and the last capture.

$$Daily\ growth = \frac{MCL_f - MCL_i}{d}$$

Daily growth was log transformed to normalize the data set and subsequently analyzed using a linear mixed-effect model [lme4 package in R (Bates *et al.*, 2013)]. We examined the relationship between MCL at hatching and daily growth in the first-year post-hatching by setting log-transformed daily growth as the dependent variable and MCL at hatching as an independent fixed effect. Because we used multiple individuals from the same clutch in this analysis, we included maternal identification number as a random effect. We also included precipitation treatment as a random effect to control for the effect of rainfall on offspring growth. Further interpretation of the effect of rainfall on daily growth was beyond the scope and intent of this study.

Results

Heavier animals were larger and offspring size was also generally correlated with maternal size. Mass and MCL were correlated in both adult females (r = 0.71, *n* = 24, *P* < 0.01; Fig. 1a) and in juveniles (r = 0.74, *n* = 120, *P* < 0.01; Fig. 1b). Over 2 years, 29 clutches produced offspring (2011 *n* = 10, 2012 *n* = 19), with five mothers contributing clutches in both years. Mean offspring MCL positively correlated with maternal MCL ($\beta = 0.11$, SE = 0.06, $P = 0.05$; Fig. 2). There was no demonstrable relationship between mean offspring MCL and clutch size ($\beta = 0.02$, SE = 0.02, $P = 0.35$), nor was there any indication of an interactive effect between maternal MCL and clutch size ($\beta = 0.02$, SE = 0.02, $P = 0.34$). Larger mothers thus generally produced larger offspring, but offspring length was not negatively affected by increasing clutch sizes.

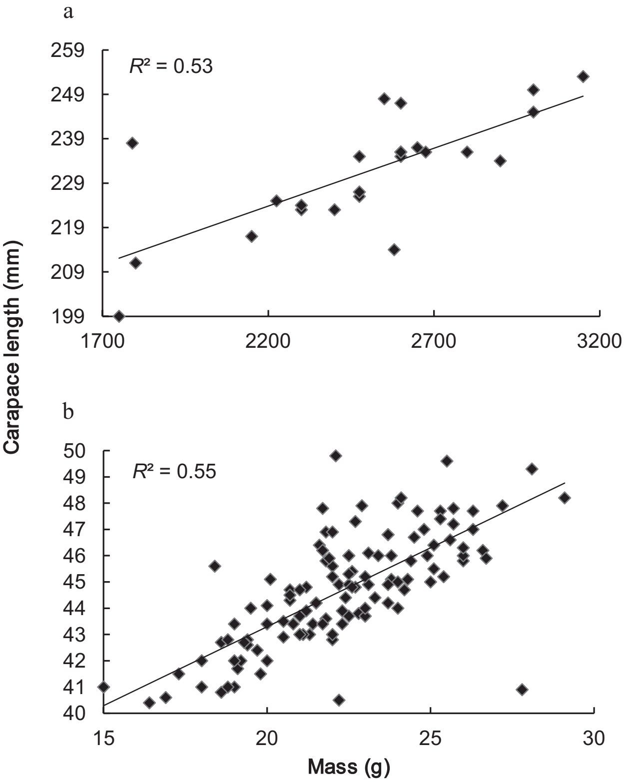

Figure 1 The relationship between mid-line carapace length (MCL) and mass in adult female (a) and hatchling (b) desert tortoises. In both adults and juveniles, carapace length (mm) was positively correlated with mass (g).

Of the 2011 nests, 39 hatchlings successfully emerged from 41 eggs (95% hatching success) and post-hatching survival was 100% during the first year. Of the 2012 nests, 81 hatchlings emerged from 102 eggs (79% hatching success). Seven hatchlings emerged during a flood and were found dead within hours after emerging from nests in 2012, reducing hatching success to 72 hatchlings (70.5%). Twenty-four of the 72 hatchlings in 2012 were excluded from the survival analysis because they were released from captivity as part of a separate study of their spatial ecology. Of the remaining 48 juveniles kept in outdoor pens that comprise this study, 87.5% survived their first year. Bodies of five of the six mortalities were not recovered. The juveniles were assumed to have died because they were never seen in a year of searching. First-year survival was positively correlated with MCL at hatching ($\beta = 1.1$, SE = 0.5, $P = 0.04$; Fig. 3). For every 1 mm increase in initial MCL, the

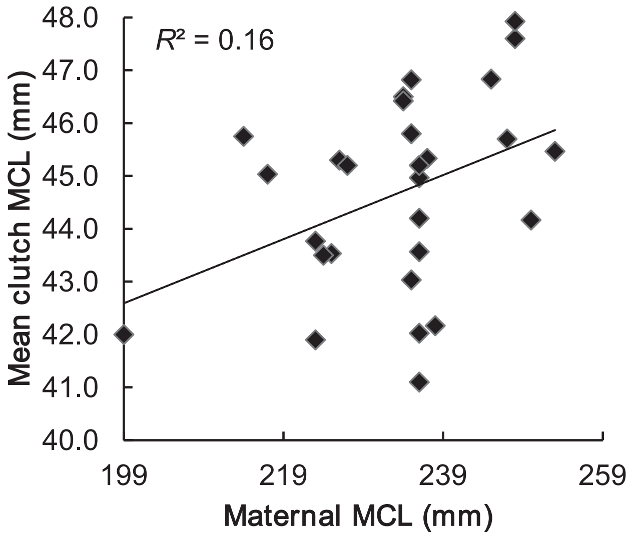

Figure 2 The relationship between mean clutch mid-line carapace length (MCL; mm) and maternal carapace length (mm) for each clutch in 2011 ($n = 10$) and 2012 ($n = 19$). Offspring carapace length was positively correlated with maternal MCL across years.

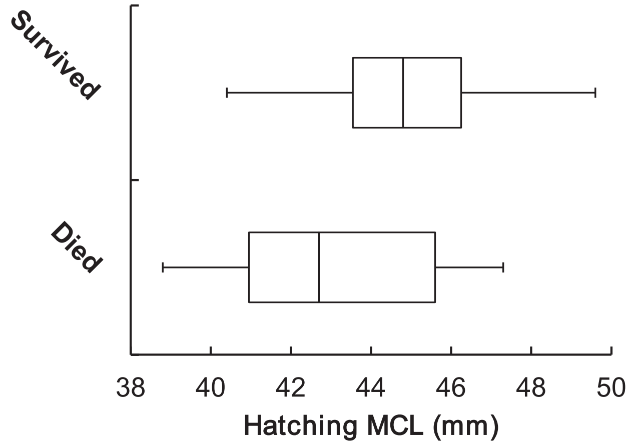

Figure 3 Size distribution of hatchlings that survived or died within their first year after hatching. Individuals with larger mid-line carapace lengths (MCL) tended to have greater survival than individuals that emerged from eggs with smaller MCLs. The box represents the lower through upper quartiles, with the mean depicted by the line contained within the box. Whiskers depict the total range from smallest to largest individuals that lay outside the upper and lower quartiles.

odds of a hatchling surviving its first year doubled. In sum, larger carapace size at hatching correlated with first-year survival in the absence of predation pressure.

Individuals from the 2011 nests grew on average 0.03 ± 0.01 mm per day ($\mu \pm 1$ SD), with most animals increasing in length by over 70% in the first year after hatching. Growth by hatchlings in their first year was strongly positively correlated with MCL at hatching ($\beta = 0.007$, SE $= 0.002$, $P < 0.001$). In other words, hatchlings with longer carapace lengths grew relatively more rapidly than shorter hatchlings.

Discussion

We found that offspring size in Mojave Desert tortoises increased with maternal size and was correlated with growth and survival for at least 1 year after hatching. Offspring size and maternal size are often correlated in vertebrates; larger fish (Kindsvater, Rosenthal & Alonzo, 2012), lizards (Olsson & Shine, 1997), turtles (Rowe, 1995) and mammals (Allen *et al.*, 2010) have all been shown to produce larger offspring. Our findings thus accord closely with such previous work. Our findings conflict, however, with at least one study of a different population of Mojave Desert tortoises where no association was found between hatchling and maternal carapace length (Ennen *et al.*, 2012).

The exact mechanism underlying the relationship between offspring size and maternal body size may have a number of explanations, including genetics, epigenetics, maternal nesting behavior and maternal resource availability. Body size or growth rates can be partly explained by simple heritability (Haatanen & Sorvari, 2013; Huchard *et al.*, 2014); larger females may produce larger offspring because of underlying genotypes. The relationship between offspring and maternal body size may also be determined in part by mechanical limitations. One proposed hypothesis for the relationship between egg size and maternal body size is that physical limitation in egg size is a consequence of pelvic girth (Congdon & Gibbons, 1987); smaller females produce smaller offspring presumably because of constraints in their pelvic size and subsequent limits on egg size (Congdon & Gibbons, 1987). Females can also experience trade-offs between clutch size and egg size, with larger clutches yielding smaller eggs (Smith & Fretwell, 1974). We did not, however, find any interaction between clutch size and offspring body size, suggesting that for Mojave Desert tortoises, larger clutches may not result in smaller offspring.

Given the nesting behavior of turtles, there are a variety of maternal behaviors and environmental factors that likely influence offspring size and survival. Nest characteristics and incubation temperatures can cause large variation in offspring phenotypes (Gutzke *et al.*, 1987; Roosenburg & Kelley, 1996; Steyermark & Spotila, 2001). Nest incubation temperature often depends on maternal nest site selection or timing of oviposition (Brooks *et al.*, 1991; Baxter, Wilson & Morafka, 2008). The extent to which each of these factors is influenced by maternal body size is not well studied. A possible future direction for study is to examine the depth, location, temperature and humidity of nests, especially as they relate to female body size; larger females may be better able to create optimal conditions for developing embryos.

Another consideration is that the relationship between offspring and maternal carapace length is not necessarily causative and is merely correlative. A long-standing assumption is that turtles and tortoises experience indeterminate growth (Mushinsky, 2014), implying that larger individuals are typically older. However, increasing evidence supports the idea that body size in reproductive adults is explained, in part, by environmental conditions early in life (Bjorndal *et al.*, 2013; Tuberville *et al.*, 2014). In salmonids, for example, multiple phenotypes can produce the largest juveniles with the highest survival rates depending on juvenile growth rates of the mothers (Burton *et al.*, 2013). An alternative hypothesis worth consideration, therefore, is that greater fecundity, egg size or offspring size from larger mothers results from better maternal environments and actually reflects overall nutritional status rather than advanced maternal age per se.

Maternal body size was indirectly correlated with offspring fitness in our study as size at hatching correlated to subsequent growth and survival. Our finding that survival and growth positively correlated with carapace length at hatching agrees with previous work in desert tortoises that found larger animals tended to stay larger through time, but disagrees with an earlier finding that growth rates of hatchlings of different sizes were similar (Spotila *et al.*, 1994). Our results are consistent with previous work indicating initial size at hatching or birth can provide short-term advantages in growth and survival in other taxa, including other tortoises, birds and bats (Ankney, 1980; O'Brien, Robert & Tiandray, 2005; Allen *et al.*, 2010). In many vertebrates, survival increases with size, especially for juveniles. Larger juveniles can have a competitive advantage over smaller ones in territory defense and resource acquisition (Ferguson, Brown & DeMarco, 1982; Ferguson & Fox, 1984; Barboza, 1995; Tatara & Berejikian, 2012). Larger hatchlings are also frequently more mobile than smaller ones (Miller, Packard & Packard, 1987; Jayne & Bennett, 1990; Miller, 1993; Myers, Tucker & Chandler, 2007). Differences in mobility potentially affect survival and growth through foraging efficiency or via an animal's ability to escape predators. Juveniles in our study were not exposed to predation pressure because they were kept in predator-excluded pens. The survival advantage conferred by size, therefore, more likely reflected foraging efficiency, competitive advantages over smaller pen mates or other factors.

Desert tortoises live in extremely arid environments and must withstand extended periods of food and water shortages (Duda, Krzysik & Freilich, 1999). Desert tortoises, consequently, depend on large bladders that store water for metabolic maintenance. Reabsorption of water from their bladders allows for metabolism of dried forage and for maintaining osmotic balance (Nagy & Medica, 1986; Henen *et al.*, 1998; Jorgensen, 1998). Larger tortoises likely have greater water and resource storage capacity, especially given that adults are better able to sustain activity during dry seasons than are juveniles (Wilson *et al.*, 2001; Brown, Nagy & Morafka, 2005). The benefit of larger body size on survival is also commonly found in amphibians (Todd *et al.*, 2014), another group for which survival is greatly affected by water availability.

An alternative explanation for the variation in survival and growth is that the amount of energy partitioned to offspring may have varied among mothers. Larger eggs in turtles and birds often have greater lipid stores (Ankney, 1980; Congdon & Gibbons, 1985). Egg size is also correlated with hatchling size (Rowe, 1995). Larger individuals thus potentially hatch with greater energy reserves. These greater energy reserves can sustain animals during periods of resource scarcity (Bagenal, 1969; Costanzo, Lee & Ultsch, 2008). In amphibians, having more lipids at metamorphosis, a period when exogenous resource consumption ceases, increases post-metamorphic survivorship (Scott *et al.*, 2007). Greater energy reserves or reduced dependence on exogenous resources during periods of scarcity may thus be vital in improving long-term survival. Any positive effect on survival during the first few years of a desert tortoise's life, when survival is believed to be low (Ernst & Lovich, 2009), can increase overall survival and thus maternal reproductive success.

In conclusion, maternal effects in desert tortoises can have measurable consequences for offspring fitness. Larger mothers produced larger offspring; larger hatchlings in turn had greater survival and growth the first year after hatching. In long-lived species with high adult survivorship, even small improvements in juvenile survivorship can potentially change population growth rates from declining to stable (Tuberville, Gibbons & Balbach, 2009). Our work thus highlights the value of large females to populations of this declining species. Additionally, indirect effects of maternal size on offspring growth and survival may be especially relevant in harsh, uncertain environments where advantages early in life may allow animals to better grow and survive. It would be worthwhile to examine the strength of this relationship in populations across the continuum of the Mojave Desert, where varying precipitation patterns and resource variability may shape the relative value of maternal effects in early life.

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