

The influence of propagule size and maternal nest-site selection on survival and behaviour of neonate turtles

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Summary

1. Maternal effects are widely recognized as important ecological and evolutionary forces, with fitness consequences for both offspring and parents. However, little is known about the impact of maternal effects on the survival and behaviour of offspring in the field. Two potentially important maternal effects that influence the phenotypes and survival of neonates are (i) propagule size effects (i.e. egg size, hatchling size and clutch effects) and (ii) nest-site selection, which influences incubation conditions and the suitability of habitat for neonates after hatching.

2. We experimentally removed the effects of variable incubation conditions to focus on how propagule size effects and nest-site microhabitat characteristics influence hatchling survival and behaviour in the Common Snapping Turtle, *Chelydra serpentina*. Using eight simultaneous experimental releases of neonates under ecologically relevant field conditions, we detected no influence of propagule size effects on hatchling turtle survival or behaviour. Egg mass, hatchling mass, hatchling carapace length and clutch did not predict the probability of survival, nor did they influence hatchling behaviour.

3. However, nest-site microhabitat characteristics were important for both survival and behaviour. The probability of hatchling survival increased with decreasing amounts of ground vegetation and slope at the release point. Hatchlings took longer to reach the fence when released in areas with more ground vegetation and dispersed farther when released from greater distances and at lower slopes. Therefore, variation in maternal behaviour, such as nest-site selection, is an important effect because it results in differential survival and behaviour of offspring in this population, whereas variation in maternal size effects alone does not.

Key-words: *Chelydra serpentina*, maternal behaviour, maternal effects, offspring survival

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Introduction

Natural selection acts on numerous phenotypic traits during early life-history stages, as few neonates ever reach adulthood. At the same time, if maternal contributions to variation in offspring phenotypes exist, then maternal effects may be subject to natural selection as well. A wide variety of maternal effects exist that could result in differential fitness of offspring: examples include propagule size and number, hormone or nutrient provisioning of offspring and pre- and posthatching maternal behaviour (Bernardo 1996a; Mousseau & Fox 1998). However, a key issue is

whether the observed variation attributable to a maternal condition or behaviour actually results in fitness differences among offspring.

Propagule size is a well-known and often-studied maternal effect in a wide variety of taxa (reviewed in Bernardo 1996b). Larger eggs in animals usually result in offspring with increased survival (reviewed in Azevedo *et al.* 1997). Most studies of hatchling turtles indicate that larger hatchlings have a survival advantage (Janzen 1993; Janzen *et al.* 2000a; but see Congdon *et al.* 1999). In addition to propagule size, other maternal effects such as behaviour may affect offspring phenotypes and survival. One such behaviour, nest-site selection, influences nest success and offspring phenotypes in many organisms, including insects, birds and reptiles (e.g. Rausher 1983; Temple 1987; Shine & Harlow 1996; Martin 1998; Wilson 1998; Hatchwell *et al.* 1999; Sandeghi & Gilbert 1999; Kolbe & Janzen 2002). Therefore, nest-site selection provides a tangible

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way to investigate the fitness consequences of variation in maternal behaviour. Subsequent monitoring of offspring survival and behaviour provides a strategy for evaluating the fitness consequences of this maternal effect. However, most studies of nest-site selection have focused on nest success (e.g. Dunk *et al.* 1997; Hatchwell *et al.* 1999); studies documenting the effect of maternal behaviour on the survival of individual offspring are lacking (but see Andrews *et al.* 2000).

Oviparous reptiles are excellent subjects for studies of maternal effects because of the variety of ways in which mothers can influence their offspring. Egg size varies within and among turtle species (Congdon & Gibbons 1985), and a positive relationship between clutch/egg size and female size exists in many species (e.g. Congdon *et al.* 1987). Through nest-site selection, mothers influence the incubation conditions experienced by developing embryos, which has a profound impact on offspring phenotypes. Body size, sex, developmental rate, locomotor behaviour, thermoregulation behaviour, growth rate and other offspring traits are all influenced by incubation conditions (e.g. Miller *et al.* 1987; Packard *et al.* 1987; Plummer & Snell 1988; Janzen *et al.* 1990; Shine & Harlow 1996; O'Steen 1998; Packard *et al.* 1999). In addition to hatchling phenotypes, nest-site selection also influences hatchlings after they have emerged from the nest by determining the habitat through which they migrate. Furthermore, for most oviparous reptiles, incubation conditions are not confounded with parental care as in birds, mammals, or viviparous reptiles, allowing for a direct evaluation of maternal effects without the need to control for parental care.

Many natural populations of turtles are characterized by low survivorship during the first year of life, a period that includes migration from nest to water (e.g. Iverson 1991; Congdon *et al.* 1994; Cunnington & Brooks 1996). The probability of surviving from emergence to 1 year of age was only 9% for a Snapping Turtle population in Michigan, USA (Congdon *et al.* 1994). Survivorship of neonates in release experiments varies substantially, ranging from 11 to 75% for three different species (Janzen 1993; Congdon *et al.* 1999; Tucker & Paukstis 1999; Tucker 2000). Thus, focusing on the early life-history phase of migration from nest to water is warranted because of the potential for strong selection pressure to act on traits influencing neonatal survivorship.

In this study, we focused on the influence of two maternal effects – propagule size and nest-site selection – on neonate survival and behaviour in Common Snapping Turtles, *Chelydra serpentina* L. We experimentally removed incubation conditions as a source of variation in offspring size with a common garden design. This approach allowed us to dissociate the effects of initial egg size and nest microhabitat from the effects of incubation conditions on postemergence survival and behaviour of hatchlings. Previous studies clearly connect incubation conditions and hatchling

body size in both the laboratory and the field (e.g. Packard 1999; Packard & Packard 2000). However, studies addressing offspring survival have not always distinguished between the effects of size and condition related to maternity and those related to incubation conditions (e.g. Congdon *et al.* 1999). Additionally, the effects of microhabitat on survival and behaviour of hatchlings during migration have not been considered previously.

Materials and methods

NEST MICROHABITAT CHARACTERISTICS

We collected eggs from fresh Snapping Turtle nests on 27–28 May 1998 from National Wildlife Refuge and US Army Corps of Engineers land along the Mississippi River in Whiteside and Carroll Cos., IL, USA. We quantified four microhabitat characteristics for each nest: distance to the water, ground vegetation, slope and aspect. We first measured the perpendicular distance from each nest to the bank of the river. Subsequently, ground vegetation was cut in four 0.25 × 0.25 m quadrats placed 1 m from the centre of each nest in each cardinal direction. The samples were dried to a constant mass and the mean weight of the four samples gave the dry vegetation mass (= ground vegetation) for each nest. Samples were taken 1 m from the centre of the nest because the vegetation was disturbed by nesting females such that measures representative of prenest conditions could not be obtained. Finally, slope was measured with an inclinometer and aspect with a compass directly over the nest site. These microhabitat characteristics were chosen because of their potential influence on incubation conditions and as cues for females in nest-site selection (e.g. Schwarzkopf & Brooks 1987; Roosenburg 1996; Kolbe & Janzen 2002). The same four microhabitat characteristics were measured at release points (RPs) to allow comparison of microhabitat data from natal nests and RPs. This comparison was necessary to determine if natal nests and the RPs we chose had similar microhabitat characteristics.

EGG INCUBATION

We collected 624 eggs from 12 nests (mean clutch size = 52.0, SD = 3.5) and marked each egg individually for future identification. Eggs were kept in moist sand in Styrofoam coolers in the shade until transportation to the lab on 29 May 1998. In the lab, eggs were weighed to the nearest 0.01 g and assigned randomly to one of 26 plastic boxes with moistened vermiculite at a water potential of –150 kPa (338 g water to 300 g vermiculite). As clutch size permitted, two eggs from each clutch were placed in each box. Boxes were rotated three times a week to minimize the effects of undetected thermal gradients in the environmental

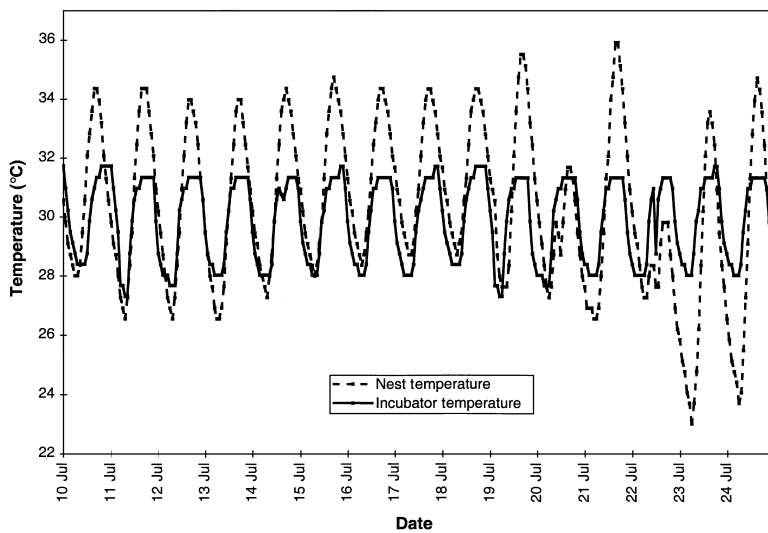


Fig. 1. A 2 week comparison of temperatures from the centre of a 1998 naturally incubated *Chelydra serpentina* nest at the release site and those from inside an egg box in one of the incubators used in this study. Mean nest temperature and the amplitude of daily cycles varied substantially between nests, but the period of the daily cycle in the incubators was similar to that in natural nests. Temperatures were recorded every 72 min with HOBO® XT temperature loggers (Onset Computer Corporation, Pocasset, MA, USA).

chambers (Bull *et al.* 1982) and were rehydrated once weekly to maintain the initial water potential. Two incubators were used, each housing 13 boxes.

The daily incubation regime consisted of 12 h 28 °C/ 12 h 31 °C. Fluctuating temperatures were chosen to provide a daily temperature regime more like natural nests (e.g. Packard *et al.* 1985; Cagle *et al.* 1993). Incubation temperatures were similar in their daily cycle to natural nest temperatures at our field site in 1997 and 1998, and were within the range of mean nest temperatures at this site over the past 2 years (range = 26.3–34.1 °C) (Fig. 1). Constant temperatures within this range produce all females in this population (Janzen 1995; O'Steen 1998). One voucher specimen from each clutch was sexed; all 12 were female, so sex was not a confounding factor in this experiment. This common garden laboratory design allowed us to control the phenotypic influence of incubation temperature, substrate water potential and environmental clutch effects on factors that would be confounded for hatchlings obtained from natural nests (see Packard *et al.* 1999; Janzen *et al.* 2000a). By minimizing differences in environmentally induced body size and condition, we could focus on the impact of propagule size effects and nest-site microhabitat characteristics on survival and behaviour. However, this design precluded us from evaluating the interaction between incubation condition (i.e. temperature and water potential) and the maternal effects of interest.

All turtles used in the release experiment hatched between 28 July and 8 August 1998. Hatchlings were weighed to the nearest 0.01 g and linear measurements

of carapace length (CL), plastron length (PL) and plastron width (PW) were taken to the nearest 0.1 mm. Turtles were housed individually in plastic cups with a moistened paper towel, covered by a lid, and were maintained under the same temperature regime as incubation. Individual identities of hatchlings were established through a combination of marginal scute notching and photocopying of plastrons (Janzen *et al.* 2000a). We marked 463 hatchlings and transported them to the field for release on 9 August 1998.

HATCHLING RELEASE

We released hatchlings from eight RPs on 11 August 1998 at the field site where the eggs were collected. We constructed a 200 × 75 m drift fence enclosure to capture hatchlings migrating down the slope to the Mississippi River and to ensure that no hatchlings left the study area (Fig. 2). One side of the drift fence was parallel to the eastern bank of the Mississippi River and ran north–south for 200 m. The fence consisted of 15-cm-high aluminium flashing supported by wooden stakes. Forty-one lidless, 25 cm high, plastic containers were buried inside the west side of the enclosure at 5 m intervals flush with the fence and sand. Four holes were punched into the bottom of each container to allow drainage.

For release of the hatchlings, we chose RPs with microhabitat characteristics similar to those of nest sites (Table 1). The eight RPs chosen were a compromise between maintaining large sample sizes for each RP (~58 hatchlings per RP) and covering the variety of microhabitats used by nesting females. During migration, hatchlings had a higher probability of experiencing microhabitat characteristics similar to those of the RP from which they were released as opposed to other RPs (Fig. 2). Artificial nests at each of the eight release points were constructed for release of each group of hatchlings. We dug an approximately 12 cm deep depression with gently sloping sides at each RP. Hatchlings were placed in the depression with a bucket over them for ~2 h. Buckets were removed between 1035 and 1100 h with a 15-m-long rope to minimize observer effects. This release time was chosen based on data from a long-term study of Snapping Turtle hatchlings in Michigan where 50% of hatchlings that emerged from natural nests were caught by 1100 h (Congdon *et al.* 1999). Collection pits were checked at 0700, 1300, and 1900 h for the duration of the experiment (1100 h on 11 August to 0700 h on 23 August = 12 days). This was the only time an observer was in the experimental release area during the experiment.

We measured three response variables in the experimental release: survival, time from release to recapture and dispersal distance. Time from release to recapture was measured as hours from release to recapture. Dispersal distance was measured as the minimum straight-line distance from the RP to the recapture pitfall trap for each recaptured hatchling.

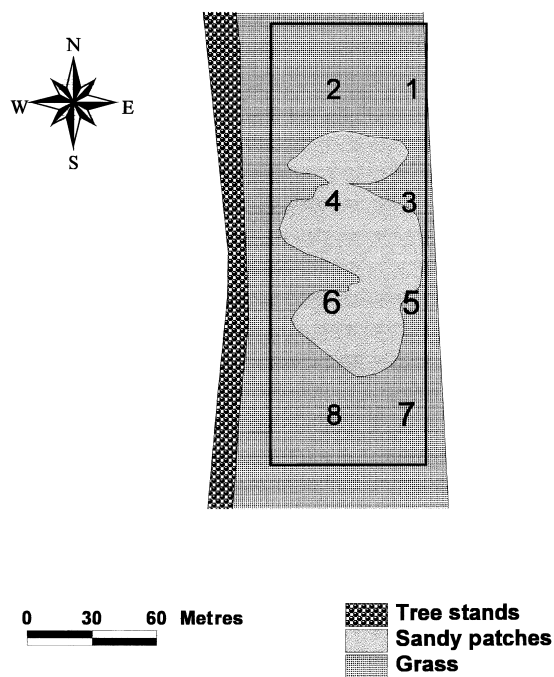


Fig. 2. Map of the study area, showing the experimental design for the hatchling release. The drift fence is depicted as the black rectangular border. Release points are numbered and correspond to Table 1. Hatchlings generally migrated down-slope from east to west towards the Mississippi River, and pit traps located along the inside of the west side of the fence recaptured them.

STATISTICAL ANALYSIS

Statistical tests were performed using JMP version 3.2 (SAS Institute, Inc. 1997). We attempted to detect any bias introduced by either incubation conditions or the experimental release. Firstly, we assessed variation in egg mass, hatchling mass and CL due to incubation conditions and release points, and then determined the proportion of variation in these phenotypes due to clutch. To accomplish this, we used split-plot ANOVAs to partition variation in egg mass, hatchling mass and CL into incubator, box within incubator, release point and clutch components. Secondly, to determine if microhabitat characteristics at RPs and natal nests were similar, we compared distance from the water, ground vegetation,

slope and aspect between RPs and nests using ANOVA. Finally, to determine if the 12 day variation in hatch date affected survival, time to recapture or dispersal distance, we used logistic and linear regression models.

To evaluate the two maternal effects (propagule size and nest-site selection), we used several independent variables for each effect. To test for a propagule size maternal effect, we used egg mass, hatchling mass, CL and clutch. Clutch was used as a composite variable, representing potential genetic, hormonal or maternal condition differences. Independent variables for the nest-site selection maternal effect were distance from the water, ground vegetation, slope and aspect. Multiple logistic regression was used to determine the probability of survival based on egg mass, hatchling mass, CL, distance from the water, ground vegetation and slope. The same independent variables representing the two maternal effects were used in multiple regression analyses to explain the two behavioral responses, time to recapture and dispersal distance. The effect of clutch (a categorical variable) on survival, time to recapture and dispersion along the fence was evaluated with chi-square tests. Aspect was analysed separately for these same dependent variables because some nests and RPs had missing values for aspect when the slope was zero (see Table 1). The analyses using clutch and aspect were corrected for multiple comparisons ($K = 3$) with the Dunn–Sidak method and gave a P value for significance of $P = 0.017$ (Sokal & Rohlf 1995).

Results

EGG INCUBATION

Of the 624 eggs initially incubated, 538 hatched (86.2%). The mean mass \pm SD for all eggs was 12.74 ± 1.96 g and the mean mass \pm SD for the eggs that produced hatchlings was 12.80 ± 1.90 g. We found no significant difference between incubators or boxes within incubator in egg mass, hatchling mass or CL for the hatchlings used in the experimental release (Table 2). Therefore, incubation conditions did not bias hatchling phenotypes in this study.

Table 1. Microhabitat characteristics of release points ($n = 8$) and natural nests ($n = 12$) in 1998. NA indicates no aspect because the slope was zero

Microhabitat characteristic	RP1	RP2	RP3	RP4	RP5	RP6	RP7	RP8	Mean				
Distance from fence (m)	70	35	70	35	70	35	70	35	52.5				
Dry vegetation mass (g)	65	28	21	6	7	1	20	31	22.4				
Slope ($^{\circ}$)	2	10	0	10	5	8	0	9	5.5				
Aspect ($^{\circ}$)	270	280	NA	270	285	270	NA	282	276.2				
	Nest 1	Nest 4	Nest 5	Nest 7	Nest 8	Nest 10	Nest 11	Nest 12	Nest 15	Nest 16	Nest 17	Nest 19	
Distance from water (m)	53	38	40	74	14	47	104	82	41	58	22	80	54.4
Dry vegetation mass (g)	7	71	95	11	14	6	103	94	77	8	81	163	60.8
Slope ($^{\circ}$)	12	13	6	5	6	14	0	0	13	4	12	3	7.3
Aspect ($^{\circ}$)	300	250	260	280	310	298	NA	NA	275	270	300	305	284.8

Table 2. *F*-ratios and levels of significance (in parentheses) for the split-plot analyses of variance performed on egg and hatchling masses of Snapping Turtles to determine the influence of incubation conditions and clutch on phenotypes. Denominator *df* = 426 for ANOVAS

Source of variation	<i>df</i>	Egg mass	Hatchling mass	CL
Incubator	1	1.57 (0.211)	1.20 (0.275)	2.47 (0.062)
Box (incubator)	24	0.94 (0.552)	0.82 (0.712)	0.99 (0.483)
Clutch	11	462.97 (< 0.0001)	422.04 (< 0.0001)	153.50 (< 0.0001)

Table 3. *F*-ratios and levels of significance (in parentheses) for the split-plot analyses of variance performed on egg and hatchling masses of Snapping Turtles to determine the influence of incubation conditions, clutch and release point on phenotypes. Denominator *df* = 426 for ANOVAS

Source of variation	<i>df</i>	Egg mass	Hatchling mass	CL
Incubator	1	7.95 (0.005)	0.65 (0.922)	0.43 (0.511)
Box (incubator)	24	0.82 (0.424)	0.67 (0.882)	0.90 (0.605)
Clutch	11	466.69 (< 0.0001)	423.90 (< 0.0001)	152.12 (< 0.0001)
Release point	7	1.75 (0.097)	1.50 (0.164)	0.72 (0.652)

RELEASE EXPERIMENT

We chose RPs similar to the original 12 nest sites in the range of values for all four microhabitat characteristics. Microhabitat characteristics of RPs and nest sites were not significantly different in distance from the water (ANOVA: $F_{1,18} = 0.03$, $P = 0.86$), ground vegetation (ANOVA: $F_{1,18} = 2.76$, $P = 0.11$), slope (ANOVA: $F_{1,18} = 0.67$, $P = 0.42$) or aspect (ANOVA: $F_{1,14} = 0.96$, $P = 0.34$) (Table 1). This design was important for determining whether natural variation in maternal nest-site selection could produce differential survival and behaviour of neonates. Additionally, there was no difference in egg mass, hatchling mass or CL among the eight RPs (Tables 3 and 4). When release point was added as a factor in the split-plot ANOVA (Table 3), we detected a significant difference in egg mass between incubators. However, the 0.03 g difference in mean egg mass between the two incubators probably did not bias any further results. There was no relationship between hatch date and survival of hatchlings during the release (logistic regression: $\chi^2 = 0.22$, $df = 1$, $P = 0.64$), time from release to recapture (linear regression: $P = 0.74$) or dispersal distance (linear regression: $P = 0.73$). Therefore, survival and migration behaviour were not affected by the 12 day variation in hatching date in the laboratory.

Overall, 291 of the 463 hatchlings released from the artificial nests were recaptured (62.9%). For the purposes of this study, only those individuals recaptured were considered as survivors. The probability of survival for those individuals not recaptured after 12 days was likely to be very low, and because of the enclosure, no hatchlings left the study area. A search of the experimental release area at the conclusion of the study did not produce any live or dead hatchlings.

Table 4. Hatchling phenotypes (CL and mass), time from release to recapture and dispersal distance for hatchlings recaptured during the experimental release of 463 hatchling *Chelydra serpentina*. Means \pm SD and ranges are reported for each release point (see Fig. 2 for corresponding RPs). The number of hatchlings released and recaptured at each RP is also given

Variable	RP1		RP2		RP3		RP4		RP5		RP6		RP7		RP8		
	Release <i>n</i> = 58	Recapture <i>n</i> = 25	Release <i>n</i> = 58	Recapture <i>n</i> = 18	Release <i>n</i> = 58	Recapture <i>n</i> = 38	Release <i>n</i> = 59	Recapture <i>n</i> = 25	Release <i>n</i> = 58	Recapture <i>n</i> = 37	Release <i>n</i> = 58	Recapture <i>n</i> = 51	Release <i>n</i> = 57	Recapture <i>n</i> = 50	Release <i>n</i> = 57	Recapture <i>n</i> = 47	
Release CL (mm)	31.3 \pm 2.3 20.4 – 35.6	31.1 \pm 1.7 26.6 – 34.3	31.5 \pm 1.7 26.7 – 35.7	31.3 \pm 1.9 25.9 – 35.4	31.4 \pm 1.7 26.8 – 35.0	31.4 \pm 1.8 25.1 – 35.0	31.3 \pm 1.9 25.9 – 35.4	31.4 \pm 1.8 25.1 – 35.0	31.4 \pm 1.7 26.8 – 35.0	31.4 \pm 1.7 26.8 – 35.0	31.4 \pm 1.8 25.1 – 35.0	31.4 \pm 1.7 26.9 – 34.9	31.4 \pm 1.7 26.9 – 34.9	31.4 \pm 1.7 26.9 – 34.9	31.4 \pm 1.7 26.9 – 34.9	31.2 \pm 1.9 25.8 – 34.9	31.2 \pm 1.9 25.8 – 34.9
Release mass (g)	10.5 \pm 1.8 6.6 – 13.8	10.4 \pm 1.5 6.7 – 13.3	10.7 \pm 1.5 6.3 – 13.1	10.6 \pm 1.5 6.4 – 13.4	10.7 \pm 1.5 6.6 – 13.4	10.6 \pm 1.6 6.2 – 13.5	10.6 \pm 1.5 6.4 – 13.4	10.6 \pm 1.6 6.2 – 13.5	10.7 \pm 1.5 6.6 – 13.4	10.7 \pm 1.5 6.6 – 13.4	10.6 \pm 1.6 6.2 – 13.5	10.6 \pm 1.6 6.2 – 13.5	10.6 \pm 1.6 6.5 – 13.5	10.6 \pm 1.6 6.5 – 13.5	10.5 \pm 1.5 6.5 – 13.5	10.5 \pm 1.5 6.5 – 13.5	10.5 \pm 1.5 6.5 – 13.5
Time to recapture (h)	141.9 \pm 59.4 50 – 272	83.3 \pm 39.7 26 – 170	70.5 \pm 32.8 26 – 164	44.0 \pm 24.2 26 – 98	63.9 \pm 25.1 20 – 98	63.1 \pm 28.8 20 – 146	44.0 \pm 24.2 26 – 98	63.1 \pm 28.8 20 – 146	63.9 \pm 25.1 20 – 98	63.9 \pm 25.1 20 – 98	76.6 \pm 39.2 20 – 260	76.6 \pm 39.2 20 – 260	76.6 \pm 39.2 20 – 260	76.6 \pm 39.2 20 – 260	62.3 \pm 30.8 26 – 152	62.3 \pm 30.8 26 – 152	62.3 \pm 30.8 26 – 152
Dispersal distance (m)	80.6 \pm 22.7 70 – 179.2	38.2 \pm 4.6 35 – 53.2	76.4 \pm 11.2 70 – 126.2	38.8 \pm 6.2 35 – 65.2	74.3 \pm 7.2 70 – 102.6	40.8 \pm 8.5 35 – 69.5	38.8 \pm 6.2 35 – 65.2	40.8 \pm 8.5 35 – 69.5	74.3 \pm 7.2 70 – 102.6	74.3 \pm 7.2 70 – 102.6	80.6 \pm 17.2 70 – 143.3	80.6 \pm 17.2 70 – 143.3	80.6 \pm 17.2 70 – 143.3	80.6 \pm 17.2 70 – 143.3	39.3 \pm 9.3 35 – 87.3	39.3 \pm 9.3 35 – 87.3	39.3 \pm 9.3 35 – 87.3

Table 5. Means \pm SD for egg and hatchling phenotypes for the 12 clutches used in the experimental release. The number of hatchlings used in the experimental release from each clutch (n) and the percentage hatchling survival for each clutch are also given

Clutch	n	Egg mass (g)	Hatchling mass (g)	Carapace length (mm)	Plastron length (mm)	Plastron width (mm)	Hatchling survival (%)
1	40	13.48 \pm 0.56	11.28 \pm 0.49	31.93 \pm 0.89	22.54 \pm 1.01	25.09 \pm 1.13	63.2
4	41	15.47 \pm 0.70	12.04 \pm 0.41	32.26 \pm 0.76	23.17 \pm 0.89	24.96 \pm 0.75	71.1
5	40	11.47 \pm 0.59	10.18 \pm 0.55	31.34 \pm 0.79	21.01 \pm 0.98	23.86 \pm 1.28	68.4
7	28	13.34 \pm 0.41	11.29 \pm 0.44	30.61 \pm 0.79	21.24 \pm 0.96	24.37 \pm 1.17	60.7
8	40	11.66 \pm 0.33	9.81 \pm 0.40	31.06 \pm 0.64	21.39 \pm 0.91	23.07 \pm 0.97	59.0
10	41	11.94 \pm 1.03	10.24 \pm 0.60	30.44 \pm 0.85	21.11 \pm 0.93	24.41 \pm 1.09	74.4
11	40	12.29 \pm 0.59	10.06 \pm 0.39	31.73 \pm 0.71	23.08 \pm 0.84	24.94 \pm 1.20	64.1
12	40	12.43 \pm 0.32	10.00 \pm 0.32	30.79 \pm 0.77	21.25 \pm 0.99	23.95 \pm 0.95	67.6
15	33	13.66 \pm 0.55	11.55 \pm 0.45	32.98 \pm 0.80	22.72 \pm 1.09	24.99 \pm 1.25	66.7
16	40	12.85 \pm 0.23	10.97 \pm 0.35	31.79 \pm 0.77	22.09 \pm 0.94	24.34 \pm 0.97	67.6
17	40	8.43 \pm 0.38	6.83 \pm 0.67	27.41 \pm 1.38	20.39 \pm 1.11	20.95 \pm 1.70	68.6
19	40	15.63 \pm 0.36	12.99 \pm 0.36	34.23 \pm 0.67	23.47 \pm 1.08	26.04 \pm 0.18	63.2

Table 6. Chi-square and levels of significance (in parentheses) for the multiple logistic regression performed to test for a maternal effect on survival. F -ratios and levels of significance (in parentheses) for multiple regressions performed on behavioural responses (i.e. time to recapture and dispersal distance) to the maternal effects are also given

Source of variation	Survival	Time to recapture	Dispersal distance
Propagule size effect			
Egg mass	0.37 (0.541)	0.36 (0.551)	1.84 (0.176)
Hatchling mass	0.12 (0.734)	1.00 (0.319)	2.09 (0.149)
CL	0.30 (0.581)	0.70 (0.787)	0.06 (0.803)
Nest-site selection effect			
RP distance	13.82 (0.0002)	0.84 (0.361)	78.16 (< 0.0001)
RP vegetation	17.98 (< 0.0001)	63.71 (< 0.0001)	0.73 (0.394)
RP slope	23.28 (< 0.0001)	0.02 (0.883)	3.63 (0.058)

PROPAGULE SIZE EFFECT

Clutches exhibited significant differences in egg mass, hatchling mass and hatchling CL (Tables 2, 3 and 5). Clutch explained over 90% of the variation in egg mass and hatchling mass and almost 80% of the variation in hatchling CL. Hatchling size varied greatly: the clutch with the largest mean size was nearly 50% heavier and 20% longer than the clutch with the smallest mean size (Table 5). Considerable variation in egg and hatchling size, comparable to that of other hatchling Snapping Turtle studies (Janzen 1993; Congdon *et al.* 1999; Packard *et al.* 1999), existed due to maternal propagule size effects alone.

The probability of survival in the release experiment was not influenced by egg mass, hatchling mass, CL (Table 6) or clutch ($\chi^2 = 2.53$; $P = 0.996$). Thus, genetic or maternal effects associated with clutch as well as additional variation in egg and hatchling body size measures did not contribute to differential survival. Furthermore, any differences in yolk quality or hormonal allocation among different clutches were not likely to influence survival. None of the measures of maternal propagule size effects influenced hatchling behaviour significantly. Egg mass, hatchling mass and CL did not have an effect on time from release to recapture or dispersal distance (Table 6). Furthermore, clutch did not have a significant effect on time

to recapture ($P = 0.033$) or dispersion along the fence ($P = 0.217$) when corrected for multiple comparisons ($P = 0.017$).

NEST-SITE SELECTION EFFECT

After assessing the maternal effect of propagule size, we evaluated the same response variables to determine if nest-site microhabitat characteristics influenced hatchling survival and behaviour. The probability of survival increased with decreasing ground vegetation and slope (Table 6). Additionally, hatchlings released from 70 m had 4% higher survival than those released from 35 m, but the biological importance of such a small survival advantage is questionable. Aspect did not have a significant effect on survival (logistic regression: $\chi^2 = 0.87$; $P = 0.352$). Therefore, some microhabitat characteristics, such as ground vegetation and slope, had strong effects on the survival of hatchlings during migration from the nest to water.

Ground vegetation, slope and distance from the fence all had significant effects on hatchling behaviour (Table 6). Hatchlings spent more time migrating to the fence when released in denser vegetation and dispersed farther when released from greater distances and at lower slopes. Aspect was not significant for time to recapture ($P = 0.044$) or dispersal distance ($P = 0.262$) when corrected for multiple comparisons ($P = 0.017$). Thus, similar microhabitat characteristics were important both for predicting the probability of hatchling survival and for influencing behaviour during migration.

Discussion

The ecological and evolutionary importance of a maternal effect hinges on the detection of fitness differences among offspring exposed to variable maternal choices or conditions. In this study, we examined the separate influence of propagule size and nest-site selection maternal effects to understand the influence of each on hatchling survival and behaviour. Egg mass, hatchling mass, hatchling CL and clutch did not

influence hatchling survival or behaviour in this experiment. However, a behavioral maternal effect, nest-site selection, influenced both the survival and behaviour of neonates. In particular, hatchlings released in less-vegetated areas and at lower slopes had a direct survival advantage over other hatchlings and a potential indirect survival advantage by taking less time to reach the fence and by travelling shorter distances (Table 6), thereby reducing their exposure to predators.

PROPAGULE SIZE EFFECT

In general, evidence exists for a positive correlation between egg size and offspring fitness components (i.e. survival, development rate and preadult size) in a wide variety of animal taxa (see Table 1 in Azevedo *et al.* 1997). However, in this study, we found no effect of egg or hatchling size on survival and behaviour (Table 6). Furthermore, clutch – a proxy for genetic or quality differences – did not contribute to survival or behaviour differences either.

In previous experimental releases of Snapping Turtle hatchlings, Janzen (1993) found that larger hatchlings had a survival advantage during migration from the nest to water and Congdon *et al.* (1999) found mixed results for the relationship between body size and survival during migration. Several methodological differences among these previous studies and this one may have contributed to these inconsistent results. Firstly, incubation conditions were different in each study. We used a common garden design with constant water potential (–150 kPa) and fluctuating temperature (28–31 °C); Janzen (1993) used two water potentials (–150 and –950 kPa) at a constant temperature (27.5 °C), and Congdon *et al.* (1999) used hatchlings from naturally incubated nests. Janzen (1993) used the two water potentials to manipulate the body size of hatchlings, with the wetter conditions producing larger hatchlings. Secondly, holding conditions after hatching and before release were different. Hatchlings in this study were kept with a moist paper towel at the same temperature as incubation; Janzen (1993) held hatchlings in 1–2 cm tap water at 20 °C, and Congdon *et al.* (1999) held hatchlings in moist soil at approximately 17 °C. Finally, microhabitats used for the release were variable in this study; Janzen (1993) used a single sparsely vegetated area similar to RPs 3–6 in this study (Fig. 2), and Congdon *et al.* (1999) did not quantify microhabitat characteristics. It is difficult to determine the exact effect of these differences in methodology, but several hatchling phenotypes, including hydration level, energy reserves and behavioural traits, may have been affected.

Although hatchling size still varied substantially in the present study due to clutch (Table 5), no size-related differences in survival or behaviour were detected. Indeed, no pattern of mortality due to maternal propagule size effects was observed in this experimental release.

NEST-SITE SELECTION EFFECT

Nest-site selection by female turtles locks eggs into a particular incubation microenvironment for the duration of their development. Hatchlings also experience particular microhabitat characteristics upon emergence and during migration to the water. We evaluated whether the survival and behaviour of hatchlings was affected by release from sites with microhabitat characteristics representative of natural nests. This experimental design addressed the importance of nest-site selection for providing an environmental context for early life-stage fitness.

In a novel finding, we showed that decreasing amounts of ground vegetation and slope had the strongest effects on increasing the probability of survival for hatchlings. Hatchlings also took less time to reach the fence when migrating through sparser ground vegetation and dispersed shorter distances when released from points closer to the fence and with steeper slopes (Tables 4 and 6). The importance of ground vegetation for hatchling survival is illustrated by the strong positive relationship between time from release to recapture and ground vegetation ($r = 0.47$, $P < 0.0001$), coupled with vegetation having a strong effect on survival. Hatchlings may migrate continuously or spend more time moving towards the water when in areas with less ground vegetation. For example, RP5 and RP6 had little ground vegetation (Table 1, Fig. 2) and even though they differed at a distance of 35 m from the fence, hatchlings from each RP took a similar amount of time to migrate to the fence (Table 4). Conversely, hatchlings in denser vegetation may move more slowly because of a reduced risk of desiccation; they may also encounter more obstacles to continuous movement, or an elevated risk of predation may limit the opportunity for movement. Hatchlings from the two RPs with the most ground vegetation, RP1 and RP2, took the longest time to reach the fence (Tables 1 and 4). Our results identifying the importance of ground vegetation for the survival and behaviour of hatchlings may explain why Congdon *et al.* (1999) found a significant clutch effect on survival only when hatchlings were released from their nest sites, and not when they were released directly into the marsh. Some attribute of the nest sites may have influenced hatchling survival differentially during migration to the water.

In addition to hatchling survival, nest-site selection at this site was also influenced by ground vegetation (Kolbe & Janzen 2002). Female turtles selected nest sites with shorter vegetation, more open sand and fewer cacti than random locations. Successful nests (i.e. those nests that escaped predation) were located more often in areas with sparse ground vegetation compared with random locations. The same ground vegetation characteristics that define nest sites and successful nests also affect nest temperatures and offspring sex ratios (Kolbe & Janzen 2002). Therefore,

ground vegetation is an important microhabitat characteristic for female nest-site selection, nest success, nest temperature and sex ratios and hatchling survival during migration from nest to water.

POTENTIAL SOURCES OF HATCHLING MORTALITY

Although the sources of hatchling mortality were not identified in this study, two likely causes during migration from the nest to water are predation and desiccation. A series of field experiments (Janzen *et al.* 2000a,b) on the survival of neonate Red-eared Slider turtles showed that avian predation induced selection favouring larger hatchlings. Hatchling survival increased from 34.9% in the control experiment to 72.4% when avian predators were excluded. Directional selection favouring larger hatchlings was detected in the control experiment, but no selection was detected when avian predators were excluded (Janzen *et al.* 2000b). These experiments lend strong support for selection on body size (or correlated traits) by avian predators, but the potential for other predators and environmental conditions to act as selective forces has not been shown. Although a variety of avian, mammalian and reptilian predators were observed in the general area of the release during this study, and many others are known to occur in the area (Janzen 1993), we did not observe any predation events or predators within the enclosure. If avian predation was non-existent or weak in the current study, then size-dependent mortality may not be expected.

Some evidence exists for the influence of hydration on hatchling survival and behaviour. Snapping Turtle hatchlings from eggs incubated on wet substrates have greater water contents, both absolutely and proportionally, than those incubated on dry substrates. Furthermore, hatchlings with greater water content are more resistant to desiccation and lose more water before their performance is affected (Finkler 1999). Therefore, under an environmental (e.g. high temperature or low humidity) selective agent, hatchlings from eggs incubated on wet substrates will probably have a survival advantage. In a release of hatchling Red-eared Sliders incubated under two slightly different moisture conditions, hatchlings from wetter incubation substrates were more likely to be recaptured than those from drier incubation substrates (Tucker & Paukstis 1999). Additionally, Finkler *et al.* (2000) found that Nebraska Snapping Turtle hatchlings from wet incubation treatments moved greater distances and spent a greater proportion of the distance travelled in low-vegetation areas than dry-treatment hatchlings. Thus, hatchlings with proportionally more water content may be better able to withstand higher temperatures and lower relative humidity during migration. Both hatchling size and hydration may be the targets of selection depending on the situation: predators may select on body size and environmental conditions may select on hydration. Studies manipulating the hydration

states of hatchlings prior to release (Tucker & Paukstis 1999; Filoramo & Janzen 2002) or using populations that have evolved under different temperature and humidity conditions (Finkler *et al.* 2000) may provide valuable insight into the consequences of hydration state for survival and behaviour. A range of weather conditions occurred during the 12 days of the experiment, including hot, sunny days with a high temperature of 31 °C and cool, rainy days with a low temperature of 17 °C. Therefore, it is possible that desiccation accounted for some of the non-recaptures observed in this study; however, the common garden incubation probably minimized variation in hydration to some extent.

In this study, we found that a behavioural maternal effect – nest-site selection – influenced the survival and behaviour of hatchlings during their migration to the water, while propagule size maternal effects did not. Further studies showing increased survival of hatchlings released from their natal nests, instead of from sites with different microhabitat characteristics, would strengthen this conclusion in an evolutionary context. Furthermore, future studies should focus on detecting sources of mortality (e.g. Janzen *et al.* 2000b), perhaps evaluating the relative importance of predation and desiccation for hatchling survival. Finally, studies documenting the differential survival and behaviour of offspring under field conditions are critical for understanding the potential impact of maternal effects on natural populations.

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