Experimental analysis of an early life-history stage: direct or indirect selection on body size of hatchling turtles?

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Summary

1. Linking phenotype, performance and fitness has proven difficult to document despite the tremendous influence of this concept on microevolutionary research. One of the major issues involves identifying the target of selection when phenotype and performance are correlated.

2. In a replicated manipulative field experiment, we evaluated this model by testing the hypothesis that predation on hatchling turtles during the critical period of migration from their terrestrial nests to their future aquatic home is random with respect to phenotype after controlling for performance. We decoupled migration performance from body size by exposing hatchling red-eared slider turtles Trachemys scripta elegans of all body sizes to environmental conditions and potential predators for four different time durations.

3. Body size (measured at initiation of the experiment either as carapace length or as mass) of turtles that were not recovered alive was slightly smaller than that of turtles that survived the experiment. More significantly, however, the number of turtles that survived decreased with increasing exposure duration, particularly after 60 h and was unrelated to meteorological conditions.

4. Our experiment thus suggests that selective predation largely acts indirectly on body size of hatchling turtles during this important life stage, consistent with the random predation hypothesis. As enhanced migration performance minimizes exposure of larger hatchlings to predators under natural conditions upon leaving the nests, our experimental results provide strong support for the model linking phenotype, performance and fitness.

Key-words: performance, predation, survivorship, Trachemys scripta.

doi: 10.1111/j.1365-2435.2006.01220.x

Introduction

Microevolutionary analysis has been ruled by one major paradigm for the past two decades. Under this paradigm, a set of individuals with a distribution of phenotypes is viewed to yield an array of correlated performances, which in turn are linked directly to components of individual fitness (Arnold 1983). In this way, we can then account for the biological consequences of phenotypic variation in an adaptive framework (Lande & Arnold 1983). This model of phenotype–performance–fitness has guided considerable laboratory and field research and has developed our understanding of microevolution (Kingsolver & Huey 2003). Still, more frequently than not, simultaneous linkage among all three components of the model in natural populations has not been previously documented.

One approach for potentially resolving this troublesome issue is to conduct manipulative experiments (Wade & Kalisz 1990). Such experiments have been widely used in recent years, particularly by researchers interested in ecological and physiological mechanisms underlying life-history evolution in natural populations (reviewed in Sinervo & Basolo 1996; Travis & Reznick 1998; Kingsolver & Srygley 2000). This manipulative approach can be especially powerful where links between phenotype and fitness have already been documented in the field, but the intervening performance variables remain elusive.

Perhaps the most frequently examined phenotypic trait in microevolutionary studies is body size. Body size typically correlates positively with fitness (reviewed in Sinervo et al. 1992; Azevedo, French & Partridge...
Size-dependent susceptibility to predation?

1997; Janzen, Tucker & Paukstis 2000a,b), yet the performance mechanism (if any) underlying this covariation is rarely explored (Jaye & Bennett 1990; Warner & Andrews 2002; Miles 2004). For example, body size of hatchling turtles leaving the nest can be a strong predictor of recapture probability in the field when the turtles reach their future aquatic home (Janzen 1993a; Congdon et al. 1999; Tucker & Paukstis 1999; Janzen et al. 2000a,b; Tucker 2000a). In some instances, body size is a positive predictor of locomotor performance of these neonatal turtles in the laboratory (Miller 1993; Janzen 1993b) and in the field (Janzen et al. 2000a,b). A manipulative field experiment in which birds were either permitted or actively excluded from the area during hatching emergence from the nests has also clearly documented avian predation as the primary selective agent driving this size advantage during this important life stage (Janzen et al. 2000b). Yet despite all these studies, the performance mechanism, if any, for the observed advantage of larger individuals during their crucial migration from nests to their future aquatic homes, is not known.

Three hypotheses are evident to explain the frequently observed lower mortality of larger neonatal turtles during migration from nest to water. First, larger hatchlings may be less likely to be preyed upon than smaller hatchlings because predators might be functionally limited in the size of turtles they can take. Second, larger hatchlings may be less susceptible to dehydration-induced mortality than smaller hatchlings. Third, larger hatchlings may spend a shorter amount of time exposed to predation than smaller ones. That is, if predation on hatchlings is random with respect to body size of the turtles and if larger individuals cover distances faster than smaller ones, then larger neonates would be more likely to survive (Congdon et al. 1999; Tucker 2000a). It is unknown whether: (1) gape-limited predator behaviour; (2) physiological limitations of turtles; or (3) size-linked performance of neonates influences the well-documented selection for larger body size of young turtles. That is, is selection acting directly on body size (options 1 or 2) or indirectly through performance (option 3)? The answer to this question can help provide general insight into the utility of the phenotype → performance → fitness framework.

We sought to test the hypothesis that predation on hatching turtles while migrating from nest to water is random with respect to body size of the animals and is thus due to indirect selection on body size mediated by size-related locomotor performance. One could tackle this issue in several ways. Most studies measure an aspect of locomotor performance of the animals under laboratory conditions and then ask statistically whether this metric relates to individual fitness in the field (e.g. Janzen 1993a). Rarely have these studies succeeded in linking laboratory performance to field fitness, probably because laboratory performance often poorly reflects field performance (Irschick et al. 2005). Alternatively, one could influence locomotor performance by physical alterations, such as adding weights. However, such alterations would then be accompanied by confounding factors (e.g. increased size). Yet another approach, which we adopt in this study, is to circumvent performance altogether and directly examine the relationship between body size and fitness.

We conducted a replicated field experiment during the period of emergence from nests of hatching red-eared slider turtles Trachemys scripta elegans (Wied 1838). Trachemys scripta is an ideal subject for this experiment for at least two reasons: (1) neonates migrate over long distances to water in large numbers after spending the winter in terrestrial nests, and (2) prior field experiments on this key life-history stage in the same well-studied natural population of this species have identified body size as a strong inverse predictor of exposure time during migration and positive predictor of recapture probability, with birds as the major cause of mortality (Tucker & Paukstis 1999; Janzen et al. 2000a,b; Tucker 2000a). We exposed neonatal turtles of all body sizes to potential predators and ambient environments for four different durations that fall within or encompass the typical 1–4-day length of the migration period. In this way, while larger turtles generally migrate from nest to water faster than smaller turtles under natural conditions (Janzen et al. 2000a,b), all turtles regardless of size or speed equally experienced predation and ambient environments for a given duration. By analogy to knockout experiments in molecular genetics, where particular genes are blocked to evaluate their functions in the larger pathways (e.g. Dellovade et al. 2000), we experimentally eliminated the possibility that typical size-linked variation in duration of exposure to predators or environmental conditions could contribute to individual fitness. Our experimental design thus permitted us to separate phenotype from performance under field conditions (i.e. by eliminating locomotor performance altogether) and thereby determine whether hatching size (direct selection) or exposure duration (indirect selection) better predicted survivorship.

Materials and methods

EGGS AND HATCHLINGS

The hatchlings used for this experiment derived from 635 clutches laid by females collected in west-central Illinois in May and June 1999. All females were collected as they attempted to nest (Tucker & Warner 1999) and oviposition was induced by intramuscular injection of oxytocin (Ewert & Legler 1978). Eggs were weighed and their length and width measured. Eggs were uniquely numbered and then incubated under ecologically relevant environmental conditions (e.g. Weisrock & Janzen 1999). The incubation substrate consisted of moist vermiculite (~150 kPa) in plastic boxes (see Tucker & Warner 1999). As the environmental chamber could not be controlled precisely, incubation temperatures varied from 25.6 °C to 28.1 °C among clutches. The
weight of all eggs and hatchlings was made with a Sartorius electronic balance to 0.01 g. All linear measurements of eggs and hatchlings were made with vernier calipers to 0.01 mm.

Clutches hatched between 1 August and 20 September 1999. Once the first egg of each clutch pipped (Gutzke, Paukstis & Packard 1984), each egg (and a small amount of vermiculite) was placed in a 120 mL Styrofoam cup. After the hatchlings emerged, they were weighed and had their straight-line carapace and plastron length measured. Once all hatchlings from a particular clutch had emerged, their plastrons were photocopied for future identification (Tucker & Paukstis 1999; Janzen et al. 2000a). Turtles were not fed during this experiment, reflecting the natural situation.

Hatchlings from all clutches were distributed into plastic boxes containing moist vermiculite (~150 kPa) for overwintering (see Janzen et al. 2000a for details). We followed this procedure before beginning the field experiment because this species overwinters in nests in west-central Illinois (Tucker 1999). Neonates were exposed to overwintering temperatures that averaged 7 °C from October 1999 to April 2000, similar to but somewhat warmer than temperatures measured in nests at the field site (Tucker & Packard 1998).

After overwintering for the typical period of approximately 7 months (Tucker 1999), 32 clutches were selected for this experiment. Each clutch contained at least 12 hatchlings that survived overwintering; overall 420 hatchlings were used. Clutches were selected following overwintering: (1) to ensure that sufficient survivors were available, and (2) to select clutches that likely contained only males due to their longer incubation periods. Overwintered clutch mates were identified individually by matching plastron patterns to photocopies made at hatching. Just prior to assigning turtles to experimental groups, they were reweighed and remeasured.

Experimental methods

Turtles from the 32 clutches were randomly assigned to 12 groups of 35 hatchlings each. These groups were then randomly assigned to one of four separate exposure times (36, 60, 84 and 108 h) such that each exposure time had three replicates. These times were chosen because it had been previously demonstrated that > 90% of hatchlings were generally recaptured within 4 days (Tucker & Paukstis 1999; Janzen et al. 2000a,b; Tucker 2000a). An additional group of 35 hatchlings served as a control. A 3 × 4 array of 12 rings was constructed from 0.3 m high aluminium flashing (Fig. 1). Each ring had a circumference of 15 m and thus a diameter of approximately 4.6 m. The array was positioned near Stump Lake, which was the site of considerable natural nesting activity and previous release experiments (see Tucker & Paukstis 1999; Janzen et al. 2000a,b for details). Exposure times were assigned to rings such that each row contained one replicate of each of the four exposure times.

Fig. 1. Diagram of array used in a selection experiment to contain hatching red-eared sliders Trachemys scripta elegans in a major nesting area of this species near Stump Lake in Jersey County, Illinois. Solid circles indicate 36 h exposure, open circles indicate 60 h exposure, circles with horizontal lines indicate 84 h exposure, circles with vertical lines indicate 108 h exposure, and circle containing “C” is the control. The two drift fences are c. 25 m apart.

A 285 m long drift fence with 20 evenly spaced pitfall traps and another 46-m long drift fence with four pitfall traps bordered the 12-ring experimental array (Fig. 1). Both fences were also constructed from aluminium flashing (Tucker & Paukstis 1999) and were separated by c. 25 m. The shorter fence was located between the array and Stump Lake to capture any experimental hatchlings that might have escaped. Finally, we constructed a 13th ring to serve as a control. This ring was covered by 0.3-cm mesh screen wire to exclude predators.

All rings and fences were installed by 6 May 2000. Old-field vegetation within rings was < 5 cm tall. We placed the turtles in the rings on 8 May at 06.00 hours after 58 mm of rainfall on 7 May, which induces emergence of hatching T. scripta from nests. Placement of experimental turtles coincided with the migration of hatchlings from natural nests to Stump Lake (Tucker 2000b).

The first group of experimental turtles was recovered at 18.00 hours on 9 May and the others at 18.00 hours on each of the next 3 days. Each ring was searched on its assigned treatment date for living hatchlings and bodies of dead hatchlings for about 30 min; the control ring was searched after 16.00 hours on 12 May (i.e. after 108 h). Living and dead turtles from all rings were identified from plastron photocopies (Tucker & Paukstis 1999; Janzen et al. 2000a). Survivors were reweighed and remeasured. Surviving turtles were released at the collecting site of the female parent. We use both carapace length and mass as measures of size (see Tucker & Paukstis 1999; Janzen et al. 2000a).

The pitfall traps on the two drift fences were checked daily at 06.00 and 18.00 hours. The shorter fence remained on site until 15 June and was checked at least
once daily, following the end of the experiment on 12 May. Only hatchlings from natural nests or a companion study (see below) were captured in the pitfall traps, thus we infer that no experimental hatchlings escaped the rings during the study. We raked the interior of each ring on 12 May to ensure that no hatchlings remained hidden in the soil or in the low vegetation that grew inside the ring; we found no hatchlings in any ring. Maximum–minimum thermometers and a rain gauge measured environmental variables in the centre of the array.

A companion field study was conducted using the longer fence to measure exposure time during migration and survival as a function of body size in free-ranging hatchlings. Turtles were derived from different clutches of eggs incubated as described above, and were treated in the same manner as the turtles in the experiment. In this companion study, 270 turtles were released as a single group 50 m east of the 285 m drift fence on 30 April (Fig. 1). The pitfall traps along this fence were checked twice daily for these animals until 16 May as described above. Consequently, this study permits us to evaluate whether temporal variation in selection (i.e. different conditions at the site during prior field experiments compared with this study) might have contributed to results obtained in the focal experiment reported here.

**STATISTICAL ANALYSES**

Measures and weights prior to release for all turtles and those made after removal for surviving hatchlings, were available for analysis. Before addressing our primary hypotheses, we first made certain that the body size distributions of the turtles in each ring of the experimental array were similar. To do so, we initially performed two nested analyses of variance in JMP version 5.1.2, one on carapace length at release and the other on body mass at release, using treatment as a fixed effect and replicate nested within treatment as a random effect. In both cases (and for subsequent similar statistical analyses of mass at recovery and mass lost for recaptured hatchlings), replicate was not a significant factor (*P* ≥ 0.19 in all four analyses), thus we re-analysed the data for those four variables without replicates. As the size distributions at release were not significantly different (see Results), we had confidence that our starting points were similar for each ring of the experimental array.

Turtles were initially classified as either: (1) recaptured alive; (2) found dead; or (3) not found. Total numbers in each category were determined for each of the 12 experimental rings in the array. However, because no experimental hatchlings were found to have escaped, all the turtles in the control ring survived the entire length of the study, and no turtles were found upon raking the rings at the end of the experiment, it is reasonable to assume that predators consumed the missing turtles in the experimental rings (see also detailed statistical assessments of these survival categories in Janzen *et al.* 2000a,b; which support this assumption). Consequently, we combined the ‘found dead’ and ‘not found’ categories for all statistical analyses.

We initially asked if hatchling size at release was related to ensuing survival status in our experiment independent of performance (i.e. independent of duration of exposure). If so, then we expected that body size at release should vary between hatching recapture categories regardless of exposure duration and, based on prior research, that hatchlings that were larger at the initiation of the field experiment would be more likely to survive until the end of the study. We evaluated this directional hypothesis by comparing carapace length and body mass at release between the two recapture categories using one-tailed one-way analyses of variance in JMP.

We subsequently asked if predation was random with respect to hatchling size and thus if exposure time during migration is typically a substantial predictor of neonatal survival during this important life stage. If so, then we expected that time of exposure rather than (or more so than) hatching size would affect survivorship. We tested this hypothesis by comparing the influence of hatching size, exposure duration, their interaction, and replicate nested within exposure duration on survivorship using nominal logistic regression analyses in JMP.

Finally, we asked if exposure time and survivorship both covaried with body size of free-ranging hatchlings as detected in previous experiments at the field site. If so, then we expected larger hatchlings to reach the drift fence sooner and have a higher probability of being recovered alive than smaller hatchlings. We tested the first hypothesis by evaluating the correlation between body mass at release and date of recapture for turtles recovered alive. We tested the second hypothesis by comparing the relationship between body mass at release and survivorship using logistic regression. We also used a logistic regression approach to calculate a linear selection gradient for body mass at release (Janzen & Stern 1998) for comparison with prior release experiments and to guide interpretation of our recapture results in the focal experiment.

**Results**

Daily rainfall and maximum and minimum temperatures over the course of the experiment are listed in Table 1. American crows *Corvus brachyrhynchos* (Brehm 1822), red-winged blackbirds *Agelaius phoeniceus* (Linnaeus 1766), and common grackles *Quiscalus quiscula* (Linnaeus 1758) were observed inside experimental rings, apparently foraging, but were not in any noticeably higher concentration than outside the rings. Neither carapace length at release nor mass at release varied significantly among the four time durations (carapace length: *d.f.* (3, 416), *F* = 0.66, *P* = 0.57; mass: *d.f.* (3,
Table 1. Environmental conditions during a selection experiment with hatching red-eared sliders Trachemys scripta elegans at a site near Stump Lake, Jersey County, Illinois conducted in May 2000

<table>
<thead>
<tr>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>8</td>
<td>0-01</td>
<td>22-1</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>14-3</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>6-6</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>15-4</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>20-9</td>
</tr>
</tbody>
</table>

416, $F = 0·62, P = 0·60$) or between the control and the duration treatments ($P > 0·11$ in both cases; see Table 2 for size summaries for the control and for the four time durations tallied across replicates).

All 35 control turtles were recovered alive at the end of the experiment. However, of 420 experimental turtles, 172 (41%) were recovered alive, 200 (47%) were not found, and 48 (11%) were found dead inside the rings (Table 2). Only turtles from natural nests were found in the two drift fences outside the rings, so it is likely that no experimental turtles escaped from our array.

Body size of experimental turtles at release did not differ, or differed weakly, between recapture categories when ignoring duration of exposure to predators and prevailing environmental conditions. Mass at release of nonsurviving experimental turtles (mean ± standard error, range = 5·98 ± 0.06, 3·68–8·96 g) and those recaptured alive (6·09 ± 0.07, 4·16–8·13 g) did not differ significantly [d.f. (1, 419), $F = 1·40$, one-tailed $P = 0·12$]. However, carapace length at release differed slightly between those two recapture categories (30·32 ± 0·12, 24·9–35·3 mm and 30·64 ± 0·15, 26·0–35·0 mm, respectively) [d.f. (1, 419), $F = 2·81$, one-tailed $P = 0·05$]. In addition, Shapiro–Wilks tests show that carapace length at release was normally distributed in both recapture categories ($W = 0·99, P = 0·1209$ and $W = 1·00, P = 0·8358$, respectively). Thus, smaller turtles, on average, may have been more likely to die than to be recovered alive. Nonetheless, the overall results did not strongly support the hypothesis that larger turtles were more likely to survive than smaller turtles, as observed in prior experiments at the site that instead used free-ranging hatchings (e.g. Janzen et al. 2000a,b).

Statistical analyses revealed a substantial effect of exposure duration on change in body mass during the experiment. Surviving turtles exposed for longer durations weighed less [d.f. (3, 168), $F = 6·64, P = 0·0003$] and lost more mass than those exposed for shorter durations [d.f. (3, 168), $F = 19·31, P < 0·0001$] (Table 2).

Exposure time was significantly associated with mass at recapture ($r = -0·31, P < 0·0001$) and with mass lost since release ($r = +0·52, P < 0·0001$), apparently reflecting mass loss by metabolism and dehydration during the experiment. Heavier hatchlings at release tended to lose more mass than lighter ones ($r = +0·56, P < 0·0001$), but at a much less than isometric rate (slope = 0·27 ± 0·03).

Table 2. Descriptive statistics for turtles exposed to four different durations in a selection experiment with hatching red-eared sliders Trachemys scripta elegans at a site near Stump Lake, Jersey County, Illinois conducted in May 2000. Note that ‘mass at recovery’ and ‘mass lost’ refer only to that subset of hatchlings that was recaptured alive. Data for the last three entries are broken down by replicate

<table>
<thead>
<tr>
<th>Duration</th>
<th>36 h Mean (SD)</th>
<th>60 h Mean (SD)</th>
<th>84 h Mean (SD)</th>
<th>108 h Mean (SD)</th>
<th>Control Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>Carapace length (CL) at release (mm)</td>
<td>30·59 (1·91)</td>
<td>30·47 (1·81)</td>
<td>30·50 (2·06)</td>
<td>30·23 (1·91)</td>
<td>30·79 (1·73)</td>
</tr>
<tr>
<td>CL at release (mm) (recaptured)</td>
<td>26·0–35·3</td>
<td>25·7–34·4</td>
<td>24·9–35·0</td>
<td>25·5–34·6</td>
<td>27·0–34·2</td>
</tr>
<tr>
<td>Mass at release (g)</td>
<td>6·10 (0·92)</td>
<td>6·03 (0·88)</td>
<td>6·05 (0·97)</td>
<td>5·93 (0·93)</td>
<td>6·35 (0·80)</td>
</tr>
<tr>
<td>Mass at release (g) (recaptured)</td>
<td>4·22–8·96</td>
<td>4·05–8·36</td>
<td>3·77–8·51</td>
<td>3·68–8·64</td>
<td>4·87–7·72</td>
</tr>
<tr>
<td>Mass at release (g) (not recaptured)</td>
<td>6·09 (0·85)</td>
<td>6·07 (0·81)</td>
<td>6·32 (0·92)</td>
<td>5·80 (0·63)</td>
<td></td>
</tr>
<tr>
<td>Mass at recovery (g)</td>
<td>4·22–8·12</td>
<td>4·16–8·13</td>
<td>4·91–7·90</td>
<td>4·48–7·15</td>
<td></td>
</tr>
<tr>
<td>Mass lost (g)</td>
<td>5·32 (0·70)</td>
<td>5·07 (0·65)</td>
<td>5·02 (0·69)</td>
<td>4·58 (0·51)</td>
<td>5·75 (0·79)</td>
</tr>
<tr>
<td>Found dead</td>
<td>3·99–7·08</td>
<td>3·61–6·83</td>
<td>3·77–6·47</td>
<td>3·63–6·11</td>
<td>4·15–7·10</td>
</tr>
<tr>
<td>Not found</td>
<td>0·77 (0·28)</td>
<td>1·00 (0·35)</td>
<td>1·30 (0·41)</td>
<td>1·22 (0·42)</td>
<td>0·60 (0·27)</td>
</tr>
<tr>
<td>Recaptured</td>
<td>0·23–1·52</td>
<td>0·08–1·75</td>
<td>0·61–2·44</td>
<td>0·17–4</td>
<td>0·14–1·15</td>
</tr>
</tbody>
</table>
Such losses in mass in this experiment are likely to be unrelated to differential survival, as all of the turtles in the control ring survived. Thus, despite levels of loss of mass during this experiment that reflect those measured in prior release experiments (see Discussion), neither body size alone nor physiological capacity appear to be strongly linked to differential survival of neonates.

In contrast, eliminating differential behaviour by imposing similar exposure times for turtles of all sizes elicited strong effects on survival. Ignoring replicate for the moment, the number of turtles in the two survival categories varied significantly with the time of exposure (d.f. = 3, G = 45·4, P < 0·0001). The number of turtles not recovered alive increased with increasing exposure duration, whereas the number of turtles recaptured alive fell with increasing exposure duration (Table 2), indicating that predation occurred throughout the experimental period.

Logistic regression analyses confirmed substantial impacts of duration of exposure on survival status. The intercept of each resulting model for the two measures of body size was not significantly different from 0 ($\chi^2 = 0·26, P = 0·61$ for carapace length at release and $\chi^2 = 0·53, P = 0·47$ for mass at release). Neither carapace length at release ($\chi^2 = 0·35, P = 0·56$, mass at release ($\chi^2 = 0·86, P = 0·35$), nor their interactions with duration of exposure ($\chi^2 = 3·85, P = 0·28$ and $\chi^2 = 3·61, P = 0·31$, respectively) significantly predicted survival in these statistical models. By comparison, survivorship in the two models varied significantly among replicates within treatments ($\chi^2 = 61·17, P < 0·0001$ and $\chi^2 = 60·81, P < 0·0001$, respectively) and with duration of exposure ($\chi^2 = 30·62, P < 0·0001$ and $\chi^2 = 30·78, P < 0·0001$, respectively) (Table 2). Thus, the longer turtles were exposed to natural environmental conditions and potential predation, the fewer turtles were recaptured alive (and these turtles were marginally, but not significantly, larger at release than those that were not recovered alive).

The results of the companion experiment involving free-ranging hatchlings mirrored findings obtained in prior studies at the field site. Nearly 71% of the hatchlings were recaptured alive, c. 60% within 108 h of release. Date of recapture and mass at release were negatively correlated ($r = 0·77, P = 0·03$), indicating that heavier turtles reached the safety of the drift fence sooner than lighter turtles. Thus, heavier turtles were presumably exposed to predation for a shorter period than were lighter turtles. Accordingly, mass at release was a significant positive predictor of survival during this companion experiment ($\chi^2 = 17·78, P < 0·0001$), similar to prior studies, yielding a positive linear selection gradient ($\delta_{\text{linear}} = +0·172 \pm 0·037, P < 0·0001$). Consequently, in contrast to our focal experiment in which migration behaviour was eliminated, heavier neonates were significantly more likely to be recaptured alive because locomotor performance was permitted in our companion experiment.

**Discussion**

To our knowledge, this study is the first manipulative field experiment with any reptile or amphibian, and perhaps with any organism, designed to separate the effects of predator behaviour and neonate performance on fitness. Other researchers have studied size-dependent interspecific competitive interactions (e.g. Werner 1994) and intraspecific studies typically show that body size is an important predictor of survivorship of young or otherwise smaller animals (e.g. Jayne & Bennett 1990; Olsson 1992; Rosenberg & Pierce 1995; Smith 1996; Townsend, Akin & Jaeger 1998; Heiling & Herberstein 1999; Blomberg & Shine 2000; Warner & Andrews 2002; Wikelski & Romero 2003; Alcobendas, Buckley & Tejedo 2004). However, these studies have not identified consistent ecological and performance mechanisms behind the survivorship effect associated with larger size and performance of neonates (but see Miles 2004).

The experiment we conducted differed fundamentally from those using hatchling turtles in release experiments (i.e. Janzen 1993a; Congdon et al. 1999; Tucker & Paukstis 1999; Janzen et al. 2000a,b; Kolbe & Janzen 2001; Filoramo & Janzen 2002). In release experiments, hatchlings exit the study when they are recaptured. If a hatchling departs the release point and reaches the pits of the drift fences toward the water faster than other hatchlings do, then that individual will be exposed to terrestrial predators and environmental conditions for a shorter period of time than the other hatchlings. In such experiments, which simulate the critical terrestrial migration of neonates from their subterranean nests to their future aquatic home, if hatchling speed is positively related to body size (e.g. Janzen et al. 2000a,b), then the influence of size on survivorship of an individual cannot be separated from the influence of performance. Our experimental design takes account of differential hatchling performance because, regardless of size or speed, hatchlings were all exposed equally to predation for a given duration.

Our experiment was ecologically relevant in that we placed hatchlings in the experimental array in the midst of the annual migration of hatchlings from natural nests at the site. For instance, we caught 111 of these ‘natural’ hatchlings at the site on 7 May and 86 more ‘natural’ hatchlings on 8 May, the first day of the experiment. Thus, experimental and ‘natural’ hatchlings experienced similar predator environments and meteorological conditions during the course of our experiment. Moreover, the final percentages of hatchlings found dead (11·4%), of those recaptured alive (41·0%), and of those not found (47·6%) were similar to the percentages of the same categories in prior release experiments at this site [12·1%, 34·0% and 53·9%, respectively, in Janzen et al. (2000a) and 12·2%, 34·9% and 53·0%, respectively, in Janzen et al. (2000b)]. The duration of our experiment was also ecologically relevant because, in most of the releases conducted at the site, at least 90% of recaptured turtles were recovered.
in the first 4 days of the multiweek experiment (i.e., Tucker & Paukstis 1999; Janzen et al. 2000a,b; Tucker 2000a).

We do not know with complete certainty that hatchlings found dead and those of unknown fate were actually killed or removed by predators. However, alternative explanations seem unlikely. We found no evidence that hatchlings escaped from the experimental rings. Had that been the case, they should have been caught in the drift fence that was placed between the array and Stump Lake precisely to catch any escapees. Hatchlings that we found dead were largely dismembered with the head and internal organs removed in most cases. The fraction of turtles found dead that were ravaged by predators showed no relationship with duration of exposure (two of nine at 36 h, four of nine at 60 h, one of 10 at 84 h, and five of 20 at 108 h).

Some turtles may have succumbed to dehydration, but we found no turtles upon raking the enclosures at the termination of the experiment, and the reduction in mass of surviving hatchlings was similar to that observed in other experiments with this species at the field site (Tucker & Paukstis 1999; Janzen et al. 2000a; Tucker 2000b). More importantly, none of the control turtles died during the experiment despite being exposed to similar meteorological conditions as the experimental turtles. Thus, neither escape nor dehydration is a reasonable explanation for our results. For free-ranging turtles, the allometric relationship between mass at release and loss of mass (see Results) could lead to behaviour to minimize dehydration that interacts with duration of exposure to influence their susceptibility to predation (Kolbe & Janzen 2002). Regardless of the mechanism of death, the key point of our field experiment is that reduced exposure time maximizes the probability of survival of neonatal turtles.

In other experimental releases of hatchling turtles at this site, larger individuals were more likely to be recovered alive and reached the fence quicker than smaller ones (Tucker & Paukstis 1999; Janzen et al. 2000a,b; Tucker 2000a; our companion experiment). However, one release conducted during an unusual rainless period found no influence of hatchling size on either survivorship or time to recapture (Filoramo & Janzen 2002). Even so, the general pattern of improved survivorship of larger hatchlings during the nest to water migration period has been hypothesized to be a result of random predation with respect to turtle body size superimposed on the reduced exposure time of these larger individuals (Congdon et al. 1999; Janzen et al. 2000a,b; Tucker 2000a).

Thus, larger hatchlings are thought to be favoured by natural selection during this important life stage simply because they are exposed to predation for a shorter period of time than smaller hatchlings (Congdon et al. 1999; Tucker 2000a).

Our study strongly supports the random predation hypothesis and reveals a pattern of primarily indirect selection on body size. We found duration of exposure to predation to be the only strongly significant predictor of survivorship. At best, body size had a relatively minimal positive influence in our study, which was designed to experimentally remove the influence of size-biased performance. We did, however, observe that turtles not recovered alive were slightly smaller at the beginning of the experiment than those we recaptured alive. This observation suggests that at least some turtles that did not survive the experiment might have been victims of smaller, more gape-limited predators such as red-winged blackbirds A. phoeniceus and common grackles Q. quiscalus (see also Janzen et al. 2000b), whereas those not found may have been completely consumed by predators such as American crows C. brachyrhynchos or raccoons Procyon lotor (Linnaeus 1758), which are not gape limited (J. Tucker, unpublished work).

We can illustrate the relative fitness impacts of exposure to predation and body size by exploiting the results of both field experiments reported here. If selection acts solely on body size and not on migration behaviour, then the selection gradient for body size calculated in the companion experiment should exactly predict body size of survivors in the focal experiment where migration behaviour was eliminated. In this case, the predicted body mass of survivors in the focal experiment is given by:

\[ z^*_f = \left( \frac{\beta_{\text{grad}}}{\sigma_{\text{sic}}} \right) \cdot \sigma^2_{\text{grad}} + z^*_e, \]

where \( \beta_{\text{grad}} \) is the selection gradient obtained in the companion experiment (0·172), \( \sigma_{\text{sic}} \) is the standard deviation of body mass in the companion experiment (1·016), \( \sigma^2_{\text{grad}} \) is the variance of body mass in the focal experiment (0·854), and \( z^*_e \) is the mean body mass of all hatchlings at release in the focal experiment (6·03 g). Thus, the predicted body mass of all survivors in the focal experiment (i.e. \( z^*_f \)) is 6·17 g, but the observed value was only 6·09 g. Consequently, by this analysis, roughly two-thirds (at least) of the fitness advantage of heavier turtles in the companion experiment apparently corresponds to faster migration rates relative to lighter turtles, rather than to body mass alone.

The experimental approach that we employed permitted us to disentangle phenotype from performance in the field and thereby assess their separate contributions to individual fitness. Neonatal turtles of all body sizes were subjected to predation in the absence of differential size-related performance. That is, larger (and otherwise faster) turtles were experimentally exposed to terrestrial predation for the same length of time as smaller (and otherwise slower) turtles were. As our experiment revealed that the duration of exposure was the most important determinant of survival, we were able to show that the well-documented path between body size and fitness in neonatal turtles is primarily routed mechanistically through size-related exposure to predation under field conditions. Moreover, results from our companion experiment involving free-ranging hatchlings eliminate temporal variation in selection between studies as a possible explanation for these findings. In nature, larger hatchlings


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migrate faster than smaller hatchlings from nest to water (sensu Tucker 2000a; our companion experiment) and thereby suffer lower predation rates (this study). Therefore, our research provides explicit experimental support for the phenotype→performance→fitness paradigm. We emphasize the importance of experimental manipulations of the ecological arena in gaining a comprehensive view of natural selection and evolutionary adaptation (Wade & Kalisz 1990; Sinervo & Basolo 1996; Travis & Reznick 1998). It is under these experimental conditions that the prevailing paradigm of microevolutionary study (Arnold 1983; Lande & Arnold 1983; Kingsolver & Huey 2003) may be best realized in nature.

Acknowledgements

We thank J. Rose and J. B. Towey for assistance in collecting females and eggs, J. B. Towey for help handling and measuring hatchlings during the experiments, and R. Cosgriff for help constructing the experimental array. We are grateful to D. C. Adams, E. D. Brodie III, A. M. Bronikowski, J. H. Chick, C. W. Fox, P. C. Phillips, D. N. Reznick, members of the Janzen lab, and two anonymous reviewers for helpful comments on the manuscript or for statistical advice. This research was conducted under a scientific collecting permit from the Illinois Department of Natural Resources and was partially supported by the Illinois Natural History Survey (to J.K.T) and by National Science Foundation of the USA grants DEB9629529 and DEB0089680 (to F.J.J). This research is paper 14 of the National Great Rivers Research and Education Center.

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Received 7 February 2006; revised 18 August 2006; accepted 27 September 2006

Editor: David Reznick