Do trade-offs between predation pressures on females versus nests drive nest-site choice in painted turtles?

JEANINE M. REFSNIDER1*,†, AARON M. REEDY2, DANIEL A. WARNER3 and FREDRIC J. JANZEN1

1Department of Ecology, Evolution and Organismal Biology, Iowa State University, 251 Bessey Hall, Ames, IA, 50010-1020, USA
2Department of Biology, University of Virginia, PO Box 400328, Charlottesville, VA, 22904, USA
3Department of Biological Sciences, Auburn University, 101 Rouse Life Sciences Building, Auburn, AL, 36849, USA

*Corresponding author. E-mail: jeanine.refsnider@utoledo.edu

Received 13 May 2015; revised 6 July 2015; accepted for publication 7 July 2015

Predation strongly influences reproductive behaviours because reproducing individuals must balance mortality risks to themselves and to their offspring. In many freshwater turtles, the nest predation risk decreases with nest distance from water, whereas the predation risk to females increases farther from water. To determine whether predation pressure influences the distance from water at which female turtles nest, we measured predation pressure on nesting females and on nests, as well as the distances of nests to water, in two populations of painted turtles. Using models, we found that female survival in both populations was high and did not vary with distance from water. Nest survival was also uncorrelated with nest distance to water, although it was significantly lower than adult survival in both populations and was only 1.2% in one population. Our results suggest that nest sites are not predictably safe from predators. Instead, turtles may hedge their bets by nesting over a wide range of distances from water because any distance is risky for nests and no distance is particularly risky for the nesting female. We suggest that other factors, such as suitable incubation conditions and/or post-emergence hatchling survival, probably play a larger role than predation in driving nest-site choice in painted turtles. © 2015 The Linnean Society of London, Biological Journal of the Linnean Society, 2015, 116, 847–855.


INTRODUCTION

Predation can shape individuals’ decisions (Lima & Dill, 1990), affect population dynamics (Krebs et al., 1995; Korpimäki & Norrdahl, 1998), and drive evolution (Reznick, Bryga & Endler, 1990; Gorzelak, Salamon & Baumiller, 2012). Vulnerable life stages, such as gravid females or inexperienced juveniles, may experience particularly strong predation pressure (Shine, 1980; Kullberg & Lind, 2002). The vulnerability of both juveniles and reproducing females to predation pressure often leads to trade-offs in which females must choose between minimizing predation risk to themselves or to their offspring (insects: Courtney, 1981; Scheirs, De Bruyn & Verhagen, 2000; reptiles: Brodie, 1989; birds: Ghalambor & Martin, 2001) or to minimizing predation risk on different stages of offspring development, such as eggs vs. juveniles (insects: Pöykkö, 2006; reptiles: Kolbe & Janzen, 2001; birds: Streby et al., 2014).

In reptiles, females of many species do not care for their eggs after oviposition but, instead, rely on the micro-environment of the nest to provide adequate incubation conditions for developing embryos. Females must therefore choose nest sites that achieve specific incubation regimes maximizing offspring survival, position newly-emerged juveniles in close proximity to suitable habitat, and, in some
cases, produce specific phenotypes (Refsnider & Janzen, 2010). In species that are primarily aquatic but nest on land, the distance of a nest from water has important implications for the micro-environment experienced by developing embryos. Leatherback turtles, for example, address the opposing pressures of beach erosion risk close to the shoreline vs. hatching disorientation risk far from the shoreline by nesting at intermediate distances from the shoreline (Kamel & Mrosovsky, 2004). In painted turtles, females from southern populations nest much closer to the water than do females from more northern populations, probably because the soil closer to water has higher moisture content and thereby provides cooler incubation conditions in a warmer climate (Morjan, 2003; Refsnider et al., 2014).

Importantly, locations that are suitable for nesting may present direct costs to females, which can include heat stress (Angilletta, Sears & Pringle, 2009) or an increased risk of predation (Spencer, 2002; Spencer & Thompson, 2003). Aquatic species that nest on land, such as many turtles, exemplify the trade-off between minimizing risk to the nesting female at the same time as optimizing nest-site choice for the offspring (the ‘selfish mother hypothesis’; Schwarzkopf & Andrews, 2012). Predation on turtle nests often increases with proximity to a wetland edge (Kolbe & Janzen, 2002; Spencer, 2002; Strickland, Colbert & Janzen, 2010), possibly as a result of the linear preysearching behaviour exhibited by many terrestrial predators (Temple, 1987), which might be expected to drive females to nest farther from water. However, if predation risk to nesting females increases with distance from a wetland edge, females may favour their own survival by nesting closer to the shoreline at the expense of increased predation risk to the nest, as seen in the Murray River turtle (Spencer, 2002). Moreover, in painted turtles, older females (i.e. those with fewer future chances at successful reproduction) may accept a greater predation risk by nesting farther from water than younger females who have more future opportunities to reproduce (Harms et al., 2005).

In the present study, we investigated whether predation pressure on either nests or nesting females influences nest-site choice, in terms of nest distance to water, in painted turtles. We placed models (sensu Brodie, 1993) of nesting female turtles at different distances from wetlands during the nesting season in two populations, and measured the number of predator attacks on the models to estimate predation pressure on nesting females in each population. We also measured shortest distances of naturally-constructed nests from wetlands, and whether nests were depredated, in each population. We expected that nest distance to water would predict survival of either nests or nesting females, which would indicate that predation pressure on nests or nesting females represents a selective pressure that could influence nest-site choice in painted turtles. Alternatively, if nest distance to water does not predict nest or female survival, then nest predation per se may not be a strong driver of nest-site choice and, instead, factors such as suitability of nest incubation conditions for embryonic development or survival of emerging hatchlings may be more important.

MATERIAL AND METHODS

STUDY SITE AND SPECIES

We studied the western painted turtle, Chrysemys picta bellii, a common freshwater species that occurs primarily west of the Mississippi River from New Mexico and Arizona (Lovich et al., 2014) to southern Canada. Painted turtles live in a wide variety of aquatic habitats, from which females emerge in May and June to nest in sunny, open areas. When on land, adult painted turtles, including nesting females, are vulnerable to mammalian predators, particularly raccoons (Procyon lotor), coyotes (Canis latrans), red foxes (Vulpes vulpes), and river otters (Lontra canadensis; Ernst & Lovich, 2009). Turtle nests are depredated by a wide variety of predators, with raccoons, striped skunks (Mephitis mephitis), coyotes, thirteen-lined ground squirrels (Ictidomys tridecemlineatus), and western hognose snakes (Heterodon nasicus) acting as primary nest predators in many populations (Ernst & Lovich, 2009).

The present study was conducted within known nesting areas at two localities during the nesting season. In 2011, we collected data on nest and female predation pressure at Thomson Causeway Recreation Area, Carroll County, Illinois, located in the east-central portion of the subspecies’ range. In 2012, we repeated the study at a northern site, Tamarac National Wildlife Refuge, Becker County, Minnesota. The Illinois study site is the location of long-term research on western painted turtle nesting ecology (Schwanz et al., 2009; Warner, Jorgensen & Janzen, 2010), including previous studies on nest predation (Kolbe & Janzen, 2002; Morjan, 2003; Bowen & Janzen, 2008; Strickland & Janzen, 2010). At this site, nest predation is highest near habitat edges (Strickland et al., 2010), particularly wetland edges (Kolbe & Janzen, 2002; Spencer, 2002).

FEMALE PREDATION PRESSURE EXPERIMENT

We used models to measure predator ‘attacks’ on nesting female painted turtles (sensu Brodie, 1993). Models were constructed by covering the carapace of empty shells of painted turtles with modelling clay.
(Sargent Art) that appeared to be colour-matched to a typical painted turtle carapace. Our use of actual turtle shells ensured that models were the proper size and shape to attract predators of turtles. Turtle shells were collected in spring 2004 from the periphery of a marsh in Minnesota after a severe winterkill of painted turtles, were dry, and did not retain scent discernible to human observers. Modelling clay was applied evenly, approximately 3 mm thick, over the entire carapace and all margins of the empty shells. Marks left by predators attempting to bite the models were preserved in the clay as a record of the attack, and were used to determine the identity of predators. The clay was unaffected by rain or heavy dew, did not harden with exposure to heat or cold, and could be molded and re-smoothed after each use of an individual model. Five models of a range of sizes were used at each study site.

We used a total of ten models, varying in size from small to large adults. The same models were used at both study sites, although the placement of individual models was randomized each evening. Models were placed in the late afternoon, which is when the majority of nesting by painted turtles occurs (Ernst & Lovich, 2009), and were removed the following morning. We set models flat on the ground in sparsely-vegetated areas so they were visible from approximately 5 m away. The position and locations at which we placed models in each population closely resembled a painted turtle constructing a nest at that study site. Models were placed at 10-m intervals from the edge of a wetland starting at 0 m (i.e. immediately at the water’s edge). At the Illinois site, models were placed at 10, 20, 30 and 40 m from the wetland; high water levels in 2011 meant that 40 m was the maximum possible distance a model could be placed from a wetland or other flooded area. In Minnesota, where wetlands were more isolated from one another, we extended model placement out to 90 m, at 10-m intervals, from a wetland edge. We placed the models in different parts of the nesting area each evening to avoid acclimatizing predators to the location of models. Each morning after model placement, we scored models as ‘attacked’ if a predator’s bite imprints were left in the clay or ‘not attacked’ if no evidence of a predator attack was visible. We took photographs of every model that showed evidence of a predator attack. We also measured all incisor imprints (individual tooth width and distance between incisors) for subsequent identification of predators, and then smoothed the modelling clay over the bite imprint for reuse the next evening. We compared dimensions of tooth marks with preserved mammal skulls in the Iowa State University mammalogy collection to identify predator species.

**NEST-SITE CHOICE AND NEST PREDATION RATES**

We monitored nesting by painted turtles during May to June at each study site (2011 in Illinois and 2012 in Minnesota). Details of nesting observations are provided in Schwanz et al. (2010). Briefly, nesting females were observed from a distance to prevent disturbance. After the completion of the nesting season, nest locations were plotted in ARCVIEW (ESRI) using Cartesian coordinates established for each nest using INTERPNT (Boose, Boose & Lezberg, 1998). We used these location data to measure the distance of each nest to the water’s edge (to the nearest metre). At the Illinois site, nests were checked every 3 days for signs of predation, which generally included desiccated eggshells around an excavated hole larger and wider at the top than the hole excavated by the nesting female. In Minnesota, we covered most nests with predator-proof wire mesh but left a randomly-selected subset uncovered to estimate predation rates at that site. These uncovered nests were also checked every 3 days for signs of predation.

**STATISTICAL ANALYSIS**

All statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Cary, NC). We compared the population mean distances from water at which female turtles constructed nests using a t-test, and we used chi-square tests of association to compare nest predation rates and female predation rates between the two populations. We modelled the survival of nesting females and nests in each population using logistic regression, with distance to water as the independent predictor variable. The response variable in both models was survival, with depredated females (i.e. models receiving attacks by predators) and depredated nests coded as 0, and surviving females (i.e. models not attacked by predators) and surviving nests coded as 1.

Next, to quantify the strength of selection on nest-site choice, we calculated separate selection gradients on nest distance to water for maximizing female survival and for maximizing nest survival. We first calculated absolute fitness (i.e. survival) of each female or nest, as well as the probability of survival of each female or nest (i.e. number of surviving females or nests divided by the total number of females or nests). We then used logistic regression (Janzen & Stern, 1998) to estimate the selection gradient on nest distance to water for separate models of female or nest survival. For each population, we regressed absolute fitness of females or nests against nest distance to water, with the slopes of the regression lines representing the selection gradients (b). Finally, for each
population, we calculated the relative fitness for each female or nest during the study year as absolute fitness divided by probability of survival (Lande & Arnold, 1983). We then estimated the fitness of females that construct nests at a given distance from water as the product of female survival (based on survival of female models) and nest survival (based on survival of wild nests) at that distance in that study year. All values are reported as the mean ± SE, and statistical tests were considered significant at α = 0.05.

RESULTS

The female predation pressure experiment was conducted for a total of 60 model-nights during 1–27 June in Illinois and 180 model-nights during 8–26 June in Minnesota. We observed seven predator attacks on model females in each population, which is equivalent to a predation frequency on nesting females of 11.7% in Illinois and 3.9% in Minnesota (Pearson’s χ² = 5.0, d.f. = 1, P = 0.026). All bite marks resulting from predator attacks on models in Minnesota matched raccoon tooth dimensions (Fig. 1A). One model in Illinois was attacked by a coyote; the other six predator attacks were attributed to raccoons. In addition to the bite marks left by predators, four models in Illinois and five in Minnesota were flipped over during the attempted predation event.

We observed 174 nests in Illinois and 50 in Minnesota; 15 of the nests in Minnesota were left unprotected to assess predation rates. All but two nests in Illinois were depredated, with the two surviving nests constructed at 31 and 42 m from water. Eight of the 15 unprotected nests in Minnesota were depredated, for a nest predation frequency of 98.8% in Illinois and 53.3% in Minnesota (Pearson’s χ² = 63.1, d.f. = 1, P < 0.0001). In one case, researchers observed a nest in Minnesota being depredated by a thirteen-lined ground squirrel (J. M. Refsnider, pers. observ.) (Fig. 1B). On average, nests in Minnesota (mean 40.1 ± 24.6 m) were constructed farther from water than nests in Illinois (26.0 ± 14.3 m; t = −3.86, d.f. = 59, P = 0.0003) (Fig. 2), although this difference could be driven by the greater availability of nest sites located far from water in Minnesota compared to Illinois. To prevent differences in habitat availability from confounding results, subsequent analyses were conducted for each population separately.

Distance from water was not a significant predictor of survival of nesting females or nests in either population (all P-values > 0.30) (Fig. 3). Indeed, there was very little variation in either female survival or nest survival with respect to distance to water in either population. Female survival was high regardless of nest distance to water in both populations and, although nest survival rates also exhibited little variation with distance to water, overall nest survival was only 1.2% in Illinois. Because there was so little variation in either female or nest survival with distance to water, selection gradients on nest distance to water were negligible (Illinois, female survival: β = 0.001, nest survival: β = 0.059; Minnesota, female survival: β = 0.001, nest survival: β = 0.003). Finally, in Illinois, estimated female fitness increased with nest distance to water, whereas, in Minnesota, the relationship between female fitness and nest distance to water was very weak (Fig. 4).

DISCUSSION

When different life stages experience differential predation pressures, selection may favour individuals that optimize survival over all life stages, rather than maximize the survival of a single life stage.
Instead, females may be at equal risk of predation over the range of nest-to-water distances tested in the present study. The Illinois population had higher rates of predator attacks on nesting females than the Minnesota population, which may be a result of differences in habitat characteristics affecting the predator community. The Illinois study site is a heavily-used campground, where predators such as raccoons and skunks may be attracted to human food left at campsites, dumpsters, and fish-cleaning stations (Strickland & Janzen, 2010). Such anthropogenic attractants may increase the density of predators at the Illinois study site and, when combined with the high detectability of nesting turtles (as a result of regular mowing and removal of shrubby vegetation) and the unusually high density of nesting turtles, probably contribute to a higher predation rate of nesting turtles in Illinois. Indeed, at the Illinois study site, we have observed female painted turtles killed by predators when constructing nests (F. J. Janzen and D. A. Warner, pers. observ.). By contrast, the Minnesota site receives very little public use and therefore has fewer anthropogenic influences to attract large numbers of predators, the painted turtle population nests at much lower density, and the areas used for nesting are in more natural habitat where long grass and other vegetation more effectively conceal nesting females. It is possible that our experimental design underestimated predation pressure on female turtles because we were only able to measure predator attacks that involved bites on the models’ shells. For example, at the Illinois site, predation of nesting turtles has occasionally been observed where females are decapitated by a predator but sustain no damage to their shells (F. J. Janzen and D. A. Warner, pers. observ.). However, it is also possible that nesting females could survive a predator attack if they are bitten on the shell but do not sustain injury to soft tissue, in which case we may have overestimated mortality on nesting females as a result of predation.

In both populations, nest predation rates were considerably higher than female predation rates. Indeed, long-lived species such as turtles rely on high adult survival to outweigh low or unpredictable survival of juvenile stages and facilitate population persistence (Congdon et al., 2001). In 2011, nest predation rates in the Illinois population were almost 100%. Even taking into account the wide inter-annual variation in nest predation rates in the Illinois population (0–95%, mean 55%; Schwanz et al., 2010), the nest predation rate observed during the present study was uncharacteristically high. One explanation for this unusually high frequency of nest predation is that, in 2011, the campground in which the nesting beach was located was closed because of damage from a
spring flood. The lack of human activity during the 2011 nesting season may have attracted more nest predators than are present in other years. Nevertheless, nest predation in the present study was not predicted by the distance to water in either population, which suggests that no nest site is predictably safe from predation. Therefore, turtles may be hedging their bets by nesting over a wide range of distances from water (Fig. 2) because there is no particular distance that has a consistently lower probability of predation at which to concentrate nesting. Previous research at the Illinois study site also shows that distance to water does not always predict a nest’s predation risk. In some years, nest survival increased with distance to water, although this relationship did not hold in all years (Kolbe & Janzen, 2002). Interestingly, Illinois nests were less likely to be depredated if they were constructed near anthropogenic structures, regardless of distance to water (Strickland & Janzen, 2010). If nest survival is only correlated with distance to water in some years, then selection on females to construct nests at particular distances is likely relatively weak, as we observed in the present study.

Our results suggest that painted turtles do not select nest sites based on perceived predation risk

**Figure 3.** Survival of painted turtle (*Chrysemys picta*) nests (A, C) and nesting females (B, D) regressed on nest distance from water in Illinois (A, B) and Minnesota (C, D). Distance from water was not a significant predictor of survival of nests or nesting females in either population (all \(P\)-values > 0.30; 95% confidence intervals shown).

**Figure 4.** Population-specific relative fitness of female painted turtles (*Chrysemys picta*) nesting at varying distances to water in Illinois and Minnesota.
but, instead, may base nest-site choice primarily on other factors. For example, a nest site’s future incubation regime is a critical consideration for nesting females because incubation conditions affect offspring survival (Kolbe & Janzen, 2001; Warner et al., 2010), hatching condition and performance (Refsnider, 2013; Refsnider et al., 2013), and sex ratio in this species (Janzen, 1994; Mitchell, Warner & Janzen, 2013). Nest-site choice may also be based on survival of an additional life stage: the hatching, which must travel from the nest site to suitable habitat for development (Refsnider & Janzen, 2010). Indeed, nest-site choice affects the direction that emerging hatchlings travel to suitable habitat in the study species (Warner & Mitchell, 2013). An experimental design similar to the one used in the present study, but which also measures hatching survival as a function of nest distance to water (sensu Paitz et al., 2007), would clarify the extent to which survival of the hatching stage influences nest-site choice in turtles, particularly because the survival of the juvenile stage is known to influence nest-site choice in other taxa (Resetarits & Wilbur, 1989; Charteris, Allibone & Death, 2003; Pöykkö, 2006; Streby et al., 2014).

In both study populations, on average, turtles nesting at intermediate distances from water, which were similar to nest distances measured in the Illinois population in other years (Harms et al., 2005). Although this result supports our prediction that females would nest at intermediate distance to water, the reason may differ from what we initially hypothesized. The lack of a relationship between either female survival or nest survival and nest distance to water suggests that females cannot always predict the safety of a particular nest site, either in terms of their own predation risk or that of their nest. In Emydura macquarii, nesting females adjusted the distance from water at which they constructed nests in response to an increase in the predator population (Spencer, 2002). Birds can also adjust their nest behaviour in response to perceived predation risk (Eggers et al., 2006; Fontaine & Martin, 2006). An important avenue for future research would be to monitor the complement and relative abundance of the predator communities at our study sites during the turtle nesting season to determine whether other turtles also adjust their nesting behaviour in response to actual predation risk. If so, management of predator populations, particularly where they are artificially increased by anthropogenic activity (Hoffman & Gottschang, 1977; Congdon, Dunham & van Loben Sels, 1993; Gerht, Huber & Ellis, 2002), may be an important conservation strategy for declining freshwater turtle populations.

ACKNOWLEDGEMENTS

This study was funded by the William Clark Graduate Student Award in Ecology and Evolutionary Biology (J.M.R.) and DEB-064932 (F.J.J.). We thank the 2011 Turtle Camp Research Crew, particularly members of the Turtle Camp Research and Education in Ecology team, for dedicated data collection and the US Army Corps of Engineers for access to the Illinois site; W. Brininger, N. Powers, and H. Streby for access to and accommodation at the Minnesota site; and two anonymous reviewers for helpful comments on earlier drafts of the manuscript. Research was conducted in accordance with Institutional Animal Care and Use Committee protocols #12-03-5570-J and 6-08-6583-J (Iowa State University), Scientific Research Permits NH10.0073 (Illinois Department of Natural Resources) and 17839 (Minnesota Department of Natural Resources), and Special Use Permits 32576-OA022 and 32560-12-025 (US Fish and Wildlife Service).

REFERENCES


Spencer R, Thompson MB. 2003. The significance of predation in nest site selection of turtles: an experimental con-


