Reduced predation on roadside nests could compensate for
additional road mortality in road-adjacent painted turtle
(*Chrysemys picta*) populations

by

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Abstract

Turtles are killed on roads, yet there is little evidence of negative road effects on abundances. We hypothesized that reduced predation of roadside nests could compensate for adult road-kill. To test this, we quantified the relative differences in predation of artificial painted turtle (*Chrysemys picta*) nests near and far from roads, in a field experiment controlling for potentially confounding differences between sites. We then estimated how much adult road-kill could be compensated for by an increase in roadside nest success using Population Viability Analysis (PVA). Linearly arranged roadside nests had a 26% higher survival rate than non-road nests in a “natural” (clumped) configuration. This lead to an estimate that 3-6% annual adult road kill can be compensated for by the lower nest predation. This suggests that compensation for adult road kill via predation release is a plausible explanation for the lack of road effects on turtle abundances.

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Introduction

Many turtle species are in decline and at risk of extinction (Gibbons et al. 2000; Rhodin et al. 2018; Howell & Seigel 2019). Globally, 56% of turtle and tortoise species are threatened (Rhodin et al. 2018). Various anthropogenic and environmental factors have been implicated in these declines, including unsustainable harvesting (Gamble & Simons 2004; Schlaepfer et al. 2005; Sung et al. 2013), bycatch in inland fisheries (Dorcas et al. 2007; Bury 2011; Larocque et al. 2012; Midwood et al. 2015), disturbances associated with recreational boating (Galois & Ouellet 2007; Bulté et al. 2010) and habitat loss and degradation (Buhlmann 1995; Garber & Burger 1995; Saumure & Bider 1998).

Roads have also been identified as a cause of turtle declines (Gibbs & Shriver 2002; Gibbs & Steen 2005; Beaudry et al. 2008). This is primarily because road collisions are a major source of adult turtle mortality (Ashley & Robinson 1996; Haxton 2000; Aresco 2005; Beaudry et al. 2008). Adult turtle mortality has disproportionate effects at the population level due to turtle life history characteristics including low natural hatchling survival, delayed sexual maturity, and high natural adult survival rates (Congdon et al. 1993; Heppell 1998).

Surprisingly, there is little evidence of population-level effects of roads on turtle abundance. Dorland et al. (2014) found that proximity to high traffic roads did not reduce painted turtle (*Chrysemys picta*) population abundance. A similar result was found in a larger, multiyear study conducted on diamondback terrapin (*Malaclemys terrapin*). Road proximity did not have a significant effect on population abundance, despite frequent road mortality occurring in the area (Grosse et al. 2011). Other studies showed that

1 painted turtle populations in landscapes with high road density had similar relative abundances to those in landscapes with low road density (Marchand & Litvaitis 2004a; Steen & Gibbs 2004), or even higher relative abundance (DeCatanzaro & Chow-Fraser 2010). To our knowledge only one study has found direct evidence of negative road effects on turtle populations, for the desert tortoise (*Gopherus agassizii*; Boarman & Sazaki 2006).

Researchers have suggested various possible explanations for the lack of effect of roads on turtle abundances. If the road is relatively new, there may be no effect observed because it takes time for an increase in adult turtle mortality to translate into an observable decline in population size (Steen & Gibbs 2004). Or, the effect may be missed because turtle abundances are difficult to estimate due to very high observation error (Marchand & Litvaitis 2004a; Steen & Gibbs 2004). Alternatively, Dorland et al. (2014) speculated that their finding of no road impact on painted turtle abundance might be due to reduced predation on turtle nests along roadsides, which could compensate for effects of higher adult mortality on turtle populations near roads. Such “predation release” (Soule et al. 1988; Johnson & Collinge 2004) has been suggested to explain other cases where populations either did not change or increased in response to roads (Rytwinski & Fahrig 2007; Fahrig & Rytwinski 2009), and some support has been found for small mammals (Downing et al. 2015). Evidence to date supports the prediction that turtle nest predation is lower near roads than far from them (Hamilton et al. 2002; Marchand & Litvaitis 2004b; Langen 2009). However, there are no estimates of the amount of adult road kill that might be compensated by such a decrease in nest predation near roads. In addition, what drives turtle nest predation release near roads has not been studied.

We hypothesize that release from predation near roads could occur for three reasons. First, predator density may be lower along roads, due to road avoidance behaviors or road mortality of the predators. For example, raccoons (*Procyon lotor*)—a common freshwater turtle nest predator—forage less along roads than other edge habitats (Barding & Nelson 2008; Owen et al. 2015), which may indicate road avoidance behavior. There is also some evidence of road avoidance in another common turtle nest predator, the red fox (*Vulpes vulpes*; Baker et al. 2007), although foxes have been known to scavenge along roads (May & Norton 1996). Raccoons and foxes may also be less abundant near roads because they are frequently killed on roads (Ashley & Robinson 1996; Baker et al. 2007), which could lead to local declines in their populations (Roedenbeck & Kohler 2006).

A second potential reason for predation release near roads is that the linear spatial configuration of nests along roads could result in decreased predator search efficiency. Female painted turtles nest in areas with open canopy and well drained soils (Christens & Bider 1987), which can result in a clumped configuration of many nests in a very small area in forests, where these nesting conditions are limited (Marchand & Litvaitis 2004b; Figure 1A). In contrast, roadsides provide ample artificial nesting habitat that does not promote clumped nesting. Instead, nests have a more linear configuration resulting from the narrow, linear road shoulder habitat (Langen 2009; Figure 1B). This could result in a lower probability of a predator finding a nest near a road. A previous study using artificial turtle nests found that clumped nests were preyed upon significantly more than nests laid in a scattered configuration over a larger area (Marchand et al. 2002), likely due to lower predator search efficiency. It follows that linear nests may also experience a lower rate of predation than clumped nests.

Finally, predation release along roadsides could result from an interaction of risk-reduction behavior of predators and reduced search efficiency. If predators perceive the lack of cover and presence of vehicles at roads as a risk (Frid & Dill 2002), they may respond to that risk by reducing the time spent foraging for nests near roads (Brown 1999; Lima & Bednekoff 1999). This effect could result in a lower nest predation rate for linear nests than clumped nests along roads because more time and effort is required to search for linear nests. However, away from roads, the predator can safely spend more time searching for nests, which could result in similar predation rates for linear and clumped nests away from roads.

Here we tested our three hypotheses for how roads could result in release from turtle nest predation, using a design that distinguished between them. Specifically, we used a field experiment to test the predictions that (1) nest predation rates are lower for roadside nests than non-roadside nests, (2) nest predation rates are lower for linearly placed than clumped nests, and (3) nest predation rates are lower for roadside, linearly placed nests than any other road-adjacency and configuration combinations. To test these predictions we used artificial turtle nests in a replicated block study design where each block contained 4 sites, one each of the 4 combinations of road adjacency (“Road” or “No-Road”) and nest configuration (“Linear” or “Clumped”), i.e. Road-Linear, Road-Clumped, No-Road-Linear and No-Road-Clumped.

We then used the rates of nest predation obtained from our artificial nest experiment in a Population Viability Analysis (PVA) to estimate the rate of adult road kill that could be compensated for by the increase in nest success near roads in a population over time. For predation release to compensate for adult turtle road mortality, the increase in population

size due to reduced nest predation would need to match the decrease in population size caused by adult road mortality.

Methods

Nest Predation Experiment

Experimental design

We simultaneously tested the road adjacency, nest configuration and interaction predictions using a replicated block design with 15 blocks. A single block contained 4 sites, each representing one of the 4 combinations of road adjacency and nest configuration (Figure 2). In each block, two Road sites were centered in the gravel of the road shoulder, and two No-Road sites were approximately 1 km from the road (average 1064 m), in the forest-agriculture matrix. Within a block, the Road-Linear and Road-Clumped sites were at least 500 m apart along the road (average 861.3 m), and the No-Road-Linear and No-Road-Clumped sites were also at least 500 m apart (average 900 m). Actual distances were dictated by the locations of appropriate nest sites in each block. Ten artificial nests were built at each of the 4 sites in each block, in either a Linear configuration or a Clumped configuration, with 4 eggs per nest. Thus, in total our study included 15 blocks, 60 sites ($4 \text{ sites} \times 15 \text{ blocks}$), 600 artificial nests ($10 \text{ nests} \times 60 \text{ sites}$), and 2400 eggs ($4 \text{ eggs} \times 600 \text{ nests}$).

Note that the use of artificial nests in our replicated block design allowed us to independently test our predictions while simultaneously controlling for potentially confounding variables such as traffic and forest amount within each block (see site selection, below). Additionally, this method allowed us to control the density of nests at

each site in a way that would not be possible using real nests. That is, we could be reasonably confident that the number of nests available to predators was the same at all sites, because we specifically selected sites where turtles were unlikely to be present (see site selection, below).

We acknowledge that predation rates of artificial nests cannot be reliably compared to those of natural nests (Moore & Robinson 2004). However, artificial nests are useful tools to evaluate relative differences in predation rates between treatments. Artificial nests have been used widely for avian studies (reviewed by Major & Kendal 1996), and more recently have been used in freshwater turtle nest studies to compare predation rates between different nest treatments (Hamilton et al. 2002; Marchand et al. 2002; Marchand & Litvaitis 2004b; Strickland et al. 2010).

Site selection

We identified a candidate set of high-traffic roads in and around Ottawa, Canada (45.4215° N, 75.6972° W), using 2017 traffic data from the City of Ottawa (unpublished data) and aerial images from Google Maps street view (Google 2019). First, we used the traffic data to identify high-traffic road segments as segments between intersections with over 10000 vehicles a day. Then we used aerial images to identify the subset of high-traffic segments with gravel shoulders suitable for turtle (and artificial) nests.

This subset of high-traffic road segments was used to select candidate blocks, and sites within blocks, to control for three factors that could potentially confound tests of our predictions. First, we eliminated the potentially confounding effect of roads on nest predation at the No-Road sites by selecting high-traffic road segments with no other

roads within the adjacent 1.5 km by 2.0 km block of land. Second, we eliminated candidate blocks containing ponds or other sources of water that could have been turtle habitat. This was done to minimize the likelihood that turtles were present, so that the density of nests at our sites would be entirely determined by our experiment, without the addition of an unknown number of natural nests. In addition, this controls for variation in predation rates at different sites within a block that could be caused by differences in proximity to water, because predators of painted turtle nests preferentially forage where they expect to find nests, such as near water (Legler 1954, Christens & Bider 1987, Marchand & Litvaitis 2004b). Lastly, we controlled for potential effects of land cover on nest predation rates within each block. To do so we divided each remaining candidate block into 4 quadrants: two potential Road sites and two potential No-Road sites. The proportions of land cover within each quadrant were calculated in ArcMap 10.6 (ESRI, Redlands, California, USA) using the 2017 Annual Crop Inventory (AAFC 2017) data layer. We then calculated the difference in percent crop cover and percent forest cover between all pairs of sites within a block, and eliminated any block with a $> 30\%$ difference in either percent crop cover or forest cover between a pair of sites. This allowed us to control for land cover differences between sites, which have been found to affect nest predation rates in artificial avian nest studies (Bergin et al. 2000; Roos 2002). Both crop cover and forest cover can affect the abundances of common turtle nest predators, such as raccoons (Martin & Fahrig 2012) and foxes (Gosselink et al. 2003). The 30% cut off was dictated by the availability of suitable blocks in the study area, and left us with 29 candidate blocks.

From March to May 2019 we visited candidate blocks to request landowner permission to conduct our study on private land. We obtained permission for fifteen blocks, distributed around the Ottawa region (Figure 3). Eleven blocks were located on private land, and were accessed with landowner permission. Four blocks were located in the Ottawa Greenbelt, and were accessed with permission from the National Capital Commission (NCC).

Artificial nest construction and data collection

The artificial nest experiment took place between June 7th and July 31st, 2019. This period corresponds to the nesting season of painted turtles in the study area (Christens & Bider 1987). All 4 sites within a block were sampled on the same days, to control for variability in predator abundance or foraging behaviour across the sampling period. Groups of 10 artificial painted turtle nests were set up at each of the 4 sites in each of the 15 blocks. Nests at the Road sites were dug into the gravel of the road shoulder (Figure 4). Nests at the No-Road sites were dug into well-drained sandy or loamy soil under open canopy in fields and along trails (Figure 5). At Linear sites, nests were dug at 3 m intervals along the road (for Road-Linear sites) or along a linear transect in a field or along a trail (for No-Road-Linear sites). At Clumped sites, nest placement was semi-random, i.e. each nest was confined to the gravel shoulder (for Road-Clumped sites) or the open-canopied soil (for No-Road-Clumped sites) and within 2 m of its nearest neighbor. Clumped groupings ranged from an area of 4.7 m² to 28.6 m² (average 15.8 m²) depending on the availability of appropriate nest space provided by the road shoulder, field, or trail.

Artificial nests were built to mimic painted turtle nests following Marchand et al. (2002). To make an artificial nest, we dug a 10 cm hole into the substrate (Figure 6). We placed 4 bobwhite quail eggs, purchased from local farmers (FCQ Farms, Witty Farms, and Silver Maple Gamebirds), in each hole. Nesting turtles release bladder water (Legler 1954; Kinney et al. 1998). To mimic this, we squirted the eggs with turtle water (i.e. water that painted turtles had been living in) taken from tanks holding painted turtles at the Rideau Valley Wildlife Sanctuary. Five squirts were applied to each nest, using a squirt bottle. Displaced substrate was then pushed over the eggs, and the surface was squirted 5 more times. To minimize human scent at the nest, researchers wore disposable gloves and rubber boots, and all tools used in making the nests were placed on a tarp. Nests were not marked; locations of nests were recorded using visual landmarks and GPS coordinates.

We returned to the sites twice, 1 week and 2 weeks after nests were built, to quantify the number of nests that were predated. A nest was considered disturbed if there was any sign of digging at the nest. If a nest was disturbed but not completely predated, we counted the number of remaining eggs in the nest. We also excavated any remaining nests during the second visit to ensure we did not miss any predated nests. We judged that two weeks should be adequate to detect most predation events because actual turtle nests usually suffer the most predation within the first few days of being laid (Legler 1954; Tinkle et al. 1981; Congdon et al. 1983; Christens & Bider 1987).

Data analysis

To test our predictions, we modeled nest survival (1 = undisturbed after the 2 week sampling period, 0 = disturbed) as a function of road adjacency (Road or No-Road), nest configuration (Clumped or Linear), and the interaction of road adjacency $\times$ nest configuration, using a generalized linear mixed effects model (GLMM; binomial distribution, logit link). To account for the hierarchical structure of our study design, i.e. 10 nests per site and 4 sites per block, we also included a random effect for sites nested within blocks. We modeled nest disturbance as 0/1 and not the number of eggs destroyed per nest because the eggs remaining in disturbed nests, uncovered and exposed to the elements, would likely not remain viable (Marchand et al. 2002). Additionally, we found that eggs rarely remained undamaged when a nest was predated (see Results). We chose to model nest survival after two weeks rather than one week because we found that a substantial amount of predation occurred after the one-week check (see Results). Note, it was not necessary to include other habitat variables in the analysis because they were controlled for by the block design. We conducted this analysis in R version 3.5.2 (R Core Team 2018) using the `glmer()` function from *lme4* (Bates et al. 2015).

PVA Using Nest Predation Results

To estimate how much adult mortality could be compensated by an increase in nest success near roads, we conducted PVAs using the program VORTEX (Lacy 1993). We first established a “natural” scenario PVA, to represent painted turtle dynamics far from roads. Parameters used in the model were obtained from a painted turtle life history table synthesized by Midwood et al. (2015) from studies of various northern populations (Table 1). Juvenile and adult mortality rates, reproductive age, maximum reproductive

age and maximum age of males and females were set as fixed parameters. Demographic stochasticity was built into the model through variation in annual clutch number and size. Reproductive rate was independent of population density in the PVA because turtles appear to lack a density dependent reproductive response (Brooks et al. 1991). Carrying capacity (K) and the initial population size were set to 1023, the value used by Midwood et al. (2015) in their painted turtle PVA. The population was modeled over 500 years to take into account the long generation times of freshwater turtles (Midwood et al. 2015). To account for stochasticity in the PVA, we ran this natural scenario 100 times. At the end of the model run, we recorded mean (SD) population size after 500 years.

We then ran a “predation-release” scenario PVA to represent painted turtle population dynamics near roads. The PVA was identical to the natural scenario, with the exception that we replaced the nest predation value from Midwood et al. (2015) with a calibrated estimate derived from our nest predation experiment. We needed to calibrate our estimate because our recorded predation rates on artificial nests were much lower than those of natural nests (see Results). This was expected because our nests were placed outside of turtle habitat, such as near water, where predators of painted turtle nests would preferentially forage (Legler 1954; Christens & Bider 1987; Marchand & Litvaitis 2004b). Therefore, we could not simply substitute our observed nest mortality rates into the PVA. Instead, we scaled the mortality rates from our artificial nest experiment to those of Midwood et al. (2015). Midwood et al. (2015) used a value of 88% for the mortality rate of the 0 age class. In our nest predation experiment, the natural turtle nests (ie. No-Road-Clumped) had a 26% higher mortality rate than Road-Linear nests (the predation release configuration). Therefore, in our predation-release PVA, we decreased

the 88% 0 age class mortality rate of the Midwood et al. (2015) life table, which we used in our natural PVA, to 62%. This resulted in a 0 age class mortality rate that was scaled to the values of Midwood et al. (2015) but maintained the 26% difference between natural and predation-release nest mortality rates from our nest predation experiment.

Finally, we estimated the annual rate of adult road mortality that our observed predation release could compensate for. We had originally planned to do this by increasing the annual adult mortality rate in the predation-release PVA by increments of 1% until the mean population size of the predation-release PVA approximated the mean population size of the natural PVA. However, this method had to be altered because both the natural population and the predation-release population remained near the carrying capacity for the entire simulation period of 500 years (see Results). Therefore, to estimate the annual rate of adult road mortality that our observed predation release could compensate for, we compared the annual adult mortality for the natural and predation-release scenarios, in the situation where the populations were "barely stable" (where the population size begins to decrease over time in the PVA). To do this we first established the maximum annual adult mortality rate that the natural population could support while remaining barely stable. The PVA was run as described for the natural scenario PVA above, with the exception that here we increased the annual adult mortality rate in increments of 0.1% until the final population size was only slightly reduced after 500 years. We then did the same for the predation-release PVA, incrementally increasing adult mortality by 1%, then by 0.1%, until the population was barely stable, i.e. slightly reduced from an average of about 1000 to an average of about 800 individuals after 500 years of simulation. The difference in the estimated adult mortality rates for a barely

stable population between the natural and predation-release scenarios represented the amount of annual adult mortality that can be compensated by the reduced nest predation near roads.

To estimate the uncertainty in our estimate of the amount of adult mortality that can be compensated by reduced nest predation along roads, we repeated the same analysis as above for different values of juvenile survival and for smaller initial population sizes. Juvenile survival rates in turtles are notoriously difficult to estimate (G. Blouin-Demers, personal communications). We therefore conducted analyses with 50% higher and 50% lower juvenile mortality rates, i.e. high juvenile mortality was 75%, 45%, and 15% and low juvenile mortality was 25%, 15% and 5% for the first, second and third juvenile years respectively. Our initial population of 1023 individuals was selected based on the estimated population of Lake Opinicon, Ontario, which was the initial population size used by Midwood et al. (2015). However, painted turtle populations can be much smaller than this (Wilbur 1975; Mitchell 1988). Therefore, we conducted analyses using initial population sizes reduced by 50% and 90%, i.e. 512 and 102.

Results

Nest Predation Experiment

Over the 15 blocks, we collected nest survival data for all but one site (a Road-Clumped site) that was destroyed by road construction (Appendix A, B, C). The 10 nests in that site were removed from the analysis. We therefore collected survival data for 59 sites: 15 each of the No-Road-Clumped, No-Road-Linear and Road-Linear sites, and 14 of the Road-Clumped sites.

Our block design was sufficient to control for differences in forest and crop cover among sites within a block. There was some variation in cover among sites that could not be accounted for by the block, at most a 48.7% difference in forest cover (mean 22.8%) within a 500 m radius among sites, and at most a 42.1% difference in crop cover (mean 21.8%) within a 500 m radius among sites. However, supplementary analyses showed that this residual variation did not affect our conclusions (Appendix D).

Seventy-eight out of 590 artificial nests were predated after one week and an additional 91 nests were predated during the second week. When a nest was predated the predator(s) typically removed or damaged all 4 eggs in the nest: this was the case for 92% of predated nests. Thirty sites experienced no predation, 19 sites experienced some predation, and at 10 sites all nests were predated.

Our results supported the prediction that nest predation rates would be lower for roadside, linearly placed nests (Road-Linear) than any other treatment. Overall, Road-Linear nests experienced the least predation, with 24.7% higher survival than the next best-surviving nest treatment, and 26% higher survival than the natural treatment (No-Road-Clumped; Figure 7). Along roads, predation on Clumped nests was greater than predation on Linear nests. Away from roads, there was no appreciable difference in predation rates between Clumped and Linear nests. This resulted in a marginally significant interaction term between road adjacency and nest configuration (Table 2).

PVA Using Nest Predation Results

The results of the PVAs suggest that predation release in a road-adjacent painted turtle population can compensate for approximately a doubling in annual adult mortality in a

typical natural population, from an annual adult mortality of about 5.6% to about 10.5%. In other words, the increased nest survival rate next to roads can compensate for about a 5% additional adult mortality due to road kill (Table 3, Figure 8), 3 to 6% when considering uncertainty in the PVA (Appendix E).

Discussion

Our results support the hypothesis that predation release on turtle nests along roadsides results from an interaction of risk-reduction behavior and reduced search efficiency for nest predators foraging on roadsides, rather than road avoidance behavior or reduced search efficiency alone. This suggests that nest predators perceive roads as a risk and respond to that risk by reducing the time spent foraging for nests near roads. We suggest we found a lower nest predation rate for Linear nests than Clumped nests at Road sites because more time and effort is required to search for Linear nests. However, at No-Road sites, predators could safely spend more time searching for nests, resulting in similar predation rates for Linear and Clumped nests. Previous research has shown that some mammal species perceive roads and the disturbance associated with them (ie. noise and fast-moving objects) similarly to predation risk (Frid & Dill 2002). Additionally, our predator vigilance-efficiency explanation is consistent with some previous artificial turtle nest predation studies. Langen (2009) found that linearly placed nests experienced less predation along a highway than along a gravel road. This could occur because predators perceive lower-traffic gravel roads as less risky than higher-traffic highways, and thus spend more time foraging along a gravel road. We suggest that our explanation is also consistent with results of Hamilton et al. (2002), who found that nests, spaced 30 m apart,

had higher survival rates on roadsides than away from roads. Nest predators may be more likely to spend the time and energy to locate these highly spaced out nests away from roads than on roadsides because roads are perceived as risky.

Our results are inconsistent with those of Marchand et al. (2002) and Marchand and Litvaitis (2004b) who found that scattered artificial turtle nests were preyed on less frequently than clumped nests in the absence of roads, while we found no difference in predation rate between Linear and Clumped nests away from roads. However, in Marchand et al. (2002) and Marchand and Litvaitis (2004b) the distance between scattered nests was 30 m and the distance between clumped nests was 2 m. This would have greatly reduced predator search efficiency on the scattered nests relative to the clumped ones. In contrast, in our study Linear nests were only 3 m apart. It appears that they were close enough together for predators to find them, as long as the predators were not disturbed by traffic. Only in the roadside situation, where predators had to be more vigilant, did the wider spacing of the Linear configuration reduce predation efficiency. Thus, we speculate that the effect of linearity in reducing predation in the Road-Linear treatment was due to the more spread-out distribution of the Linear nests rather than to the Linear configuration itself.

We emphasize that our nest survival rates are not direct estimates of survival rates for actual turtle nests. Rather, they represent relative estimates for the different experimental treatments. We expected (and observed) higher survival rates than observed for real painted turtle nests in the literature. Real painted turtle nests often experience a mortality rate in the range of 80% (Christens & Bider 1987; Congdon et al. 2003), while the highest we observed was 42.9%. We expected our survival rates to be higher than natural

nests because we purposefully placed the nests in areas where we expected no actual turtle nests, i.e. far from wetlands, to avoid confounding our results with an unknown number of natural nests in the area. Therefore, predators were likely not actively searching for turtle nests in our blocks, and predation events were likely due to chance encounters. Raccoons, one of the primary painted turtle nest predators (Marchand et al. 2002), are known to learn to exploit new food sources more effectively over repeated exposure (Bowman & Harris 1980). However, this would only cause a predation release effect in this experiment if predator naivety caused larger reductions in the likelihood of encountering a nest near roads than far from them, which seems unlikely.

Artificial nests allowed us to create our experimental block design, which would have been impossible using actual turtle nests, but we acknowledge that our inferences rely on the assumption that the relative nest survival among the different treatments is representative of the relative differences in nest survival that would occur in real turtle nests if they were in those treatments. This assumption is the basis of most artificial nest studies for turtle nests (Hamilton et al. 2002; Marchand et al. 2002; Marchand & Litvaitis 2004b) and for bird nests (reviewed by Major & Kendall 1996). We note that, for artificial bird nests, this assumption is often not valid (Moore & Robinson 2004). This is often attributed to artificial bird nests lacking realism, such as when they lack the sensory cues that predators associate with real nests (Burke et al. 2005). Artificial bird nests also lack realism because they are missing the associated parental birds, which may be a cue for predators (Major & Kendall 1996). However, these problems likely do not extend to our artificial turtle nests. We used turtle water to replicate the olfactory cue associated

with turtle nests (Kinney et al. 1998), and natural turtle nests are not visited or defended by the mother turtle.

By combining the increased nest survival for the predation-release (Road-Linear) nest treatment with natural painted turtle demographics in a PVA, we estimate that increased nest survival near roads can compensate for approximately a 5% increase in annual mortality on adult turtles. As we do not have estimates of adult road kill, we do not know whether the increase in nest survival fully compensates for adult road kill in our region. However, given that Dorland et al. (2014) found no difference in population sizes near vs. far from high-traffic roads in our region, full compensation does at least seem plausible.

Nevertheless we note that our estimate of the amount of adult mortality compensated by increased nest survival is very approximate, due to uncertainty in the PVA parameter estimates. This includes uncertainty in our measured increase in nest survival. We also assumed that our relative nest mortality rates from the two-week nest predation experiment were representative of the relative nest mortality rates for the whole 0 age class in the PVAs. We suggest this is a reasonable assumption because the majority of the 0 age class mortality occurs during this 2-week period. This is because most nest predation occurs within the first few days of laying (Legler 1954; Tinkle et al. 1981; Congdon et al. 1983; Christens & Bider 1987), and in the northern parts of the painted turtle's range hatchlings remain in the nest and do not emerge until the following spring, effectively remaining in the nest for their first year of life (Christens & Bider 1987). Thus, the predation experienced during the first two weeks likely encompasses the majority of predation events for the year. We also note that even if this assumption is

incorrect and there is substantial mortality after the 2-week period, this should not change our conclusions, if we can assume that the *relative* mortality rates among the 4 treatments remain after the 2-week period.

There is also uncertainty in the other PVA parameters, which we took from Midwood et al. (2015). These values were based on painted turtle populations in Michigan, Virginia and Pennsylvania. Ideally, they would have been collected from populations in our study region as population parameters do vary throughout the range of painted turtles (Ernst 1971; Wilbur 1975; Congdon & Tinkle 1982; Mitchell 1988; Congdon et al. 2003). We estimated the influence of uncertainty, by varying the two values most poorly known for turtle populations in our area, namely juvenile survival and population size. Results suggest that the percent adult mortality compensated for by predation release can be relatively insensitive to changes in PVA parameters. For example, decreasing juvenile mortality rates in the PVA by 50% resulted in a 0.5% higher estimate of the additional adult mortality that could be compensated for by reduced nest predation (Appendix D). Increasing juvenile mortality rates by 50% reduced the estimate of the additional adult mortality that could be compensated for by reduced nest predation by 0.8%. Reducing the initial population size in the PVA by 50% also had a small effect on this estimate, with a 0.2% lower estimate of the additional adult mortality that could be compensated for by reduced nest predation (Appendix D).

We emphasize that our study does not indicate that road mortality is not a problem for other turtle species. Whether predation release can compensate for road mortality likely depends on the turtle species' life history traits. For example, a modeling study by Gibbs and Shriver (2002) predicts that large-bodied turtles can experience population declines

from road kill. This is supported by Boarman and Sazaki (2006), who found that the large-bodied desert tortoise (*Gopherus agassizii*) was less abundant close to roads. Gibbs and Shriver (2002) suggest that roads disproportionately affect large-bodied turtle species because they reach maturity later and have larger movement ranges. This should increase the relative impact of adult mortality on population persistence, relative to small-bodied species such as the painted turtle. Therefore, predation release may be less likely to compensate for adult road kill in large-bodied species. In addition, fully terrestrial species—like the desert tortoise—are much less likely to nest along roadsides than partially aquatic species, and would not benefit from road-adjacent predation release. This is because gravel roadsides do not mimic the natural nesting habitats of fully terrestrial species, which typically nest inside burrows (Lovich et al. 2014).

Predation release also might not fully compensate for adult road kill in situations where the adults must cross roads frequently, increasing the adult road kill rate. This could occur if individuals travel within a complex of multiple wetlands over the course of a year and therefore encounter more roads than populations inhabiting an isolated home pond. For example, Beaudry et al. (2008) tracked spotted turtle (*Clemmys guttata*) and blanding's turtle (*Emydoidea blandingii*) movements over roads in a dense area of small wetlands in Maine. PVAs representing the population dynamics of these populations indicated they were at risk of extirpation (Beaudry et al. 2008). Another situation where road crossings are frequent is when drought causes drying of aquatic habitat, leading to migrations. This can result in extreme rates of road mortality. For example, Aresco (2005) estimated a 98% probability of mortality for turtles crossing a four-lane highway

in Florida located between two lakes during a time of drought. It is unlikely that any level of predation release would compensate for that level of mortality.

It is important to note that roads may have other effects on turtles and their nest predators that were not evaluated in this study. It could be that road-adjacent nesting is an ecological trap for adult painted turtles. This argument has been made for nesting Blanding's turtles, because road nesting is associated with increased risk of road mortality (Refsnider & Linck 2012). However, the increased nesting success found in our study indicates that this is not the case for painted turtles as long as adult road mortality does not pass the threshold of ~5%. Roads also provide nest predators such as raccoons and foxes with road kill as an additional food source (Antworth et al. 2005; Schwartz et al. 2018). It is possible that road kill serves to subsidize nest predators, leaving them less likely to hunt for nests. However, if that were the only reason for predation-release on roads, we should have seen higher survival of Clumped nests near roads than far from them. Instead, there was slightly lower survival of roadside Clumped nests (Figure 7).

Our study adds to the growing literature suggesting that some neutral or even positive effects of roads on animal abundance may be due to predation release. Rytwinski and Fahrig (2013) conducted a simulation study which suggested that populations of small species are often unaffected by road density, and positively affected when their generalist predators are negatively affected by road density. This is reflected in the empirical literature, as positive effects of roads are common for small mammal species (Johnson & Collinge 2004; Rytwinski & Fahrig 2007; Bissonette & Rosa 2009; Ruiz-Capillas et al. 2013). Downing et al. (2015) found that white-footed mice (*Peromyscus leucopus*) experience less predation from birds in areas of high road density. Additionally, white

tailed deer have been found to benefit from increased road density, which could be due to predation release (Munro et al. 2013).

We emphasize that a positive effect of roads on prey populations via predation release also represents a negative effect of roads on the predator species, through road kill (Ashley & Robinson 1996; Grilo et al. 2009), road avoidance (Baker et al. 2007; Barding & Nelson 2008; Owen et al. 2015), or reduced predation success (Kerley et al. 2002). In fact, predators may be particularly vulnerable to road effects because of their low reproductive rates and wide-ranging movement (Rytwinski & Fahrig 2011). Large predator species, such as wolves and bears, have been found to suffer negative road effects (Popp & Donovan 2016). Nest predation release is likely just one of a series of species interactions occurring in this system. If predation release on painted turtle nests laid on roadsides is part of a larger trophic cascade, we can expect effects throughout the food chain (Wootton 1994). Future studies could be useful to determine if the positive effect of roads on painted turtle nesting success contributes to additional effects at other trophic levels.

In summary, we estimate that reduced predation of roadside nests can compensate for an estimated 5% of annual adult road kill on painted turtle populations. Thus, predation release may explain the previously documented lack of road effects on painted turtle population abundance (Marchand & Litvaitis 2004a; Steen & Gibbs 2004; Dorland et al. 2014). We reiterate that this does not constitute an overall benefit of roads. Predation release may not extend to other turtle species, and predation release on painted turtles inherently implies a negative effect of roads on their nest predators. Overall our results do

suggest the need to consider species interactions when interpreting road effects on wildlife populations.

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Tables

Table 1. Input values used for painted turtle PVA's conducted in VORTEX (Lacy 1993), taken from Midwood et al. (2015). We used PVAs to estimate the annual rate of adult road mortality that our observed estimate of predation release could compensate for. In the natural scenario PVAs, we used the Midwood et al. (2015) annual nest mortality estimate of 88%, and in the predation-release scenario PVA we used the scaled mortality rate of the Linear-Road nests from our artificial nest experiment, 62%. For both the natural and predation-release PVAs we increased the annual adult mortality rate incrementally until both populations were barely stable (population decreasing slightly over time). The difference between the annual adult mortality rates of the two barely stable populations then represents how much more annual adult mortality the predation-release population can withstand than the natural population.

Parameter	Input
Female Reproductive Age (year)	14
Male Reproductive Age	9
Max Reproductive Age	60
Max # Broods/Year	2
Max # Progeny/Brood	8
Sex Ratio at Birth	1 male : 1 female
% Adult Females Breeding	90
% Females with 0 Broods	19
% Females with 1 Broods	68
% Females with 2 Broods	13
Approximate Mean & SD for Offspring per Brood	7 (SD 2)
Mean % Age 0 Mortality/Year	natural scenario: 88.0 predation release scenario: 62.0
% Year 1 Juvenile Mortality/Year	50
% Year 2 Juvenile Mortality/Year	30
% Year 3 Juvenile Mortality/Year	10
% Adult Mortality/Year	natural scenario: 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6 predation release scenario: 6, 7, 8, 9, 10, 10.1, 10.2, 10.3, 10.4, 10.5

Table 2. Model estimates from a GLMM estimating the effect of road adjacency and nest configuration on survival of artificial turtle nests, where nest survival was coded as 1 = undisturbed after the 2 week sampling period and 0 = disturbed. Each group of 10 nests (10 nests=site; 59 sites altogether) was either adjacent to a road or approximately 1 km from a road and in either a clumped or linear configuration. (* indicates p-value <0.05, · indicates p-value <0.10).

Response Variable	Predictor Variable	Estimate	SE	z	P
Survival	Intercept	2.866	1.87	1.533	0.125
	Road Adjacency	4.252	2.078	2.046	0.041*
	Configuration	-0.492	2.303	-0.213	0.831
	Configuration : Road Adjacency	5.499	3.007	-1.829	0.067·

Table 3. Estimates of final painted turtle population size derived from PVAs for both the natural scenario (far from roads, with a clumped nest configuration) and the predation-release scenario (adjacent to roads, with a linear nest configuration). At 5% adult mortality (Midwood et al. 2015), both the natural population and predation-release population remained at the carrying capacity. We estimated the amount of adult mortality compensated by predation release, by increasing adult mortality in both the natural and predation-release populations until the populations were barely stable, i.e. a slight decline from an average of about 1000 to an average of about 800 individuals after 500 years of simulation. Barely stable populations are **bolded** in the table. The difference in adult mortality rate for the two populations (5%) was the amount of adult mortality compensated by reduced nest mortality in the predation-release population.

Scenario	% Adult Mortality	Final Population Size (SD)
natural	5	1008.05 (16.88)
natural + 0.1	5.1	1006.09 (18.95)
natural + 0.2	5.2	999.39 (25.29)
natural + 0.3	5.3	985.87 (41.26)
natural + 0.4	5.4	966.42 (52.42)
natural + 0.5	5.5	911.59 (97.04)
natural + 0.6	5.6	793.30 (144.42)
predation release	5	1021.83 (9.16)
predation release +1	6	1022.07 (8.39)
predation release +2	7	1021.49 (6.75)
predation release +3	8	1018.79 (9.79)
predation release +4	9	1013.03 (18.01)
predation release +5	10	982.60 (41.67)
predation release +5.1	10.1	977.75 (41.68)
predation release +5.2	10.2	959.73 (66.06)
predation release +5.3	10.3	928.09 (83.27)
predation release +5.4	10.4	851.64 (158.97)
predation release +5.5	10.5	793.75 (173.03)

Figures

Figure 1. (A) Natural patches of nesting habitat are characterized by open canopy and well-drained soil and can be in limited supply near ponds in forests. (B) A road shoulder near a pond provides nesting habitat for painted turtles and can reduce the concentration of nests in natural nesting patches.

Figure 2. Aerial image of one of the fifteen experimental blocks in the turtle nest predation-release experiment. Each block contained 4 artificial turtle nesting sites (10 nests in each site), representing the 4 nest treatments: Road-Linear (A), Road- Clumped (B), No-Road-Clumped (C), and No-Road-Linear (D). “Road” treatment nests were on the road shoulder, while “No-Road” treatment nests were in sandy or loamy soil under open canopy approximately 1 km away from the road. “Linear” treatments had nests placed 3 m apart along a linear transect. “Clumped” treatments had grouped nests within 2 m of their nearest neighbors. Each block was adjacent to an arterial road. The experiment was conducted from June 7th to July 31st, 2019.

Figure 3. Distribution of the 15 experimental blocks for the nest predation-release experiment (see Fig. 2) around Ottawa, Ontario, Canada. We selected high-traffic road segments with no other roads within the adjacent 1.5 km by 2.0 km block of land. We avoided blocks containing ponds or other sources of water to ensure that our artificial nests were the only nests available to predators in the area. We also selected blocks such that sites within blocks had similar percent crop cover and forest cover to control for their potential effects on nest treatments.

A

B

Figure 4. Example Clumped (A) and Linear (B) nest configurations on Road sites.

A

B

Figure 5. Example Clumped (A) and Linear (B) nest configurations at No-Road sites. No-Road sites were located in well-drained sandy or loamy soil under open canopy in fields (A) and along trails and driveways (B).

A

B

C

Figure 6. Photos of the nest building process. The nest cavity was dug to a depth of 10 cm (A). Four eggs were deposited in the nest cavity and turtle water was sprayed on them (B). The nest was covered by the displaced substrate and more turtle water was sprayed on top (C). Our turtle water was water that painted turtles had been living in, which is meant to mimic the bladder water released by nesting turtles.

Figure 7. Proportion of artificial turtle nests (total 590 nests, 10 at each of 59 sites) that survived after 14 days in each of the 4 nest treatments: Road-Clumped, No-Road-Clumped, Road-Linear and No-Road-Linear. “Road” treatment nests were built within the bounds of the road shoulder, while “No-Road” treatment nests were built in sandy or loamy soil under open canopy approximately 1 km away from the road. “Clumped” treatments are composed of grouped nests within 2 m of their nearest neighbor. “Linear” treatments are composed of nests 3 m apart along a linear transect.

Figure 8. Per capita adult and nest mortality rates for two modeled painted turtle populations, one away from roads (No-Road-Clumped; the natural scenario) and one adjacent to a road (Road-Linear; the predation release scenario). Nest mortality rates were obtained by scaling the mortality rates from our artificial nest experiment to those of Midwood et al. (2015; Fig. 2-7). We estimated the amount of adult mortality compensated by predation release by increasing adult mortality in both the natural and predation-release populations until the populations were barely stable, i.e. a slight decline from about 1000 to about 800 individuals after 500 years of simulation (Table 3). The difference in adult mortality rate for the two populations (5%) was the amount of adult mortality compensated by reduced nest mortality in the predation-release population.

Appendices

Appendix A. GPS locations, road name, average annual daily traffic, and forest and crop cover percentages of sites used in the nest predation-release experiment conducted between June 7th and July 31st, 2019. Forest and crop cover percentages were calculated both as quadrants in each 2 km by 1.5 km block, and in a 500m radius around each nest group. “Road” (Road-Adjacency = 1) treatment nests were on the road shoulder, while “No-Road” (Road-Adjacency = 0) treatment nests were in sandy or loamy soil under open canopy approximately 1 km away from the road. “Linear” (Configuration = 0) treatments had nests placed 3 m apart along a linear transect. “Clumped” (Configuration = 1) treatments had grouped nests within 2 m of their nearest neighbors.

Block	Road-Adjacency	Configuration	GPS Coordinates		Road	Average Annual Daily Traffic	Forest Cover Within Block (%)	Crop Cover Within Block (%)	Forest Cover Within 500m (%)	Crop Cover Within 500m (%)
			N	W						
1	0	1	45.14.719	075.50.004	NA	NA	2.8	96.6	4.4	94.6
1	0	0	45.14.916	075.49.593	NA	NA	1.8	97.8	0.3	97
1	1	0	45.15.418	075.49.945	Fallowfield Rd	17099	0	95.2	0.2	91.9
1	1	1	45.15.246	075.50.376	Fallowfield Rd	17099	0	97.1	0	97.3
2	0	1	45.21.387	075.33.910	NA	NA	55.1	38.8	48.8	45.4
2	0	0	45.21.060	075.34.727	NA	NA	54.6	43.9	53.4	40.2
2	1	0	45.21.568	075.35.009	Whyte Side Rd	16199	60.8	36.5	63.7	26.7
2	1	1	45.21.828	075.34.353	Whyte Side Rd	16199	44.8	50.6	45.5	48.6
3	0	1	45.10.916	075.46.473	NA	NA	8.4	82.7	8.2	80
3	0	0	45.11.113	075.45.734	NA	NA	0.1	97.9	0.2	99.8
3	1	0	45.11.675	075.46.430	Brophy Dr	17834	1.2	97.4	7	82.4
3	1	1	45.11.425	075.47.040	Brophy Dr	17834	16.6	70.2	32.5	41
4	0	1	45.17.815	075.48.333	NA	NA	77.5	0	72.2	0.6
4	0	0	45.18.230	075.48.709	NA	NA	74.3	4.3	72	4
4	1	0	45.18.041	075.49.230	Moodie Dr	17425	70	0.9	73.2	0

4	1	1	45.17.604	075.48.984	Moodie Dr	17425	55.6	0	53.1	0
5	0	1	45.28.126	075.25.159	NA	NA	0.8	93.5	0.8	87.4
5	0	0	45.27.936	075.25.608	NA	NA	7.7	82.7	7	86.6
5	1	0	45.28.506	075.26.130	Innes Rd	19339	3.3	75.6	2.7	73.5
5	1	1	45.28.715	075.25.507	Innes Rd	19339	12.4	78	14.1	70.2
6	0	1	45.26.035	075.26.789	NA	NA	5.1	76.1	6.7	80.3
6	0	0	45.26.139	075.26.351	NA	NA	0	88.2	1.9	74.2
6	1	0	45.25.494	075.26.096	Navan Rd	10196	29.2	51.7	30.1	43.6
7	0	1	45.28.585	075.24.194	NA	NA	9.5	85.8	10.9	81.8
7	0	0	45.28.769	075.23.572	NA	NA	11.6	70	6	69.3
7	1	0	45.28.993	075.24.485	Innes Rd	19339	5.5	77	6.8	78.2
7	1	1	45.29.168	075.23.854	Innes Rd	19339	18.5	75.8	7.1	84.5
8	0	1	45.18.838	075.27.452	NA	NA	22.1	63.1	12.1	74.1
8	0	0	45.19.275	075.26.723	NA	NA	45.6	40.4	38.1	46.3
8	1	0	45.19.755	075.27.033	Mitch Owens Rd	17813	47.6	49.3	38.5	51.3
8	1	1	45.19.466	075.27.751	Mitch Owens Rd	17813	30.8	46.3	12.7	51.4
9	0	1	45.15.259	075.51.762	NA	NA	0.2	96.3	0.2	94.2
9	0	0	45.14.820	075.52.334	NA	NA	0.1	96.1	0	99.9
9	1	0	45.14.501	075.51.966	Fallowfield Rd	17099	0.1	95.7	0.3	93.3
9	1	1	45.14.811	075.51.351	Fallowfield Rd	17099	0	93.5	0	93.7
10	0	1	45.11.822	075.44.475	NA	NA	7.4	86.6	3.6	90.1
10	0	0	45.11.598	075.45.011	NA	NA	25.6	65.3	30	59.6
10	1	0	45.12.047	075.45.529	Brophy Dr	17834	18.6	61.5	12.8	70.7
10	1	1	45.12.243	075.45.046	Brophy Dr	17834	23.6	59.5	25.1	47.9
11	0	1	45.11.649	075.48.051	NA	NA	7.3	88.6	8	88.2
11	0	0	45.11.497	075.48.489	NA	NA	1.7	97	3.6	93.9

11	1	0	45.10.983	075.48.164	Brophy Dr	17834	0.3	96.7	0.7	94.9
11	1	1	45.11.197	075.47.632	Brophy Dr	17834	10.4	80.4	10.3	78.9
12	0	1	45.21.917	075.35.902	NA	NA	76.6	14	47.9	36.2
12	0	0	45.21.442	075.36.439	NA	NA	90.6	3.9	96.7	0.3
12	1	0	45.21.043	075.36.190	Davidson Dr	13784	97.1	0	93.5	0
12	1	1	45.21.289	075.35.581	Davidson Dr	13784	94.5	0	95.1	0.2
13	0	1	45.09.578	075.43.288	NA	NA	28.8	66.9	34.3	61.4
13	0	0	45.09.982	075.43.561	NA	NA	5.3	89.3	4.7	87.5
13	1	0	45.10.249	075.42.883	Prince of Wales Rd	17834	6.1	72.2	6.5	62.9
13	1	1	45.09.812	075.42.643	Prince of Wales Rd	17834	5	83.4	5	80.3
14	0	1	45.19.116	075.58.373	NA	NA	14.4	56	4	60
14	0	0	45.18.598	075.57.943	NA	NA	19.8	46.2	22.8	38.8
14	1	0	45.19.035	075.57.366	Huntmar Rd	18106	10.4	62.9	8.8	64.2
14	1	1	45.19.440	075.57.859	Huntmar Rd	18106	19.3	34.4	39	24.3
15	0	1	45.19.925	075.36.714	NA	NA	64.4	20.1	40	19.4
15	0	0	45.20.129	075.37.049	NA	NA	77.1	0	79	0
15	1	0	45.19.801	075.37.755	Albion Rd	12995	89.2	0	90	0
15	1	1	45.19.550	075.37.607	Albion Rd	12995	84.2	3.7	81.5	5.2

Appendix B. Set up date, check dates, and number of artificial nests predated after 1 and 2 weeks in the nest predation-release experiment conducted between June 7th and July 31st, 2019. “Road” (Road-Adjacency = 1) treatment nests were on the road shoulder, while “No-Road” (Road-Adjacency = 0) treatment nests were in sandy or loamy soil under open canopy approximately 1 km away from the road. “Linear” (Configuration = 0) treatments had nests placed 3 m apart along a linear transect. “Clumped” (Configuration = 1) treatments had grouped nests within 2 m of their nearest neighbors. Nests were considered predated if there were any eggs missing or destroyed.

Block	Road-Adjacency	Configuration	Set Up Date & Time	One Week Check Date	Nests Predated After 1 Week	Two Week Check Date	Nests Predated After 2 Weeks
1	0	1	19-06-20 12:10	19-06-27	10	19-07-04	10
1	0	0	19-06-20 12:40	19-06-27	9	19-07-04	10
1	1	0	19-06-20 13:00	19-06-27	1	19-07-04	1
1	1	1	19-06-20 13:30	19-06-27	1	19-07-04	5
2	0	1	19-06-17 13:10	19-06-24	1	19-07-01	3
2	0	0	19-06-17 14:15	19-06-24	0	19-07-01	0
2	1	0	19-06-17 14:50	19-06-24	0	19-07-01	0
2	1	1	19-06-17 15:20	19-06-24	0	19-07-01	9
3	0	1	19-07-08 12:45	19-07-15	0	19-07-22	4
3	0	0	19-07-08 11:30	19-07-15	0	19-07-22	10
3	1	0	19-07-08 14:15	19-07-15	0	19-07-22	8
3	1	1	19-07-08 13:50	19-07-15	1	19-07-22	9
4	0	1	19-06-26 10:40	19-07-03	0	19-07-10	0
4	0	0	19-06-26 12:00	19-07-03	1	19-07-10	1
4	1	0	19-06-26 14:50	19-07-03	0	19-07-10	0
4	1	1	19-06-26 14:00	19-07-03	0	19-07-10	8
5	0	1	19-07-16 15:10	19-07-23	0	19-07-30	0
5	0	0	19-07-16 16:00	19-07-23	0	19-07-30	3

5	1	0	19-07-16 16:20	19-07-23	0	19-07-30	0
5	1	1	19-07-16 15:30	19-07-23	0	19-07-30	0
6	0	1	19-06-18 10:30	19-06-25	2	19-07-02	2
6	0	0	19-06-18 11:05	19-06-25	0	19-07-02	0
6	1	0	19-06-18 12:40	19-06-25	1	19-07-02	1
7	0	1	19-06-28 11:50	19-07-05	0	19-07-12	0
7	0	0	19-06-28 10:30	19-07-05	0	19-07-12	0
7	1	0	19-06-28 12:40	19-07-05	0	19-07-12	0
7	1	1	19-06-28 13:00	19-07-05	0	19-07-12	0
8	0	1	19-07-12 10:30	19-07-19	0	19-07-26	1
8	0	0	19-07-12 11:20	19-07-19	0	19-07-26	4
8	1	0	19-07-12 11:50	19-07-19	0	19-07-26	0
8	1	1	19-07-12 12:10	19-07-19	0	19-07-26	0
9	0	1	19-06-25 12:30	19-07-02	10	19-07-09	10
9	0	0	19-06-25 13:00	19-07-02	0	19-07-09	10
9	1	0	19-06-25 13:20	19-07-02	0	19-07-09	0
9	1	1	19-06-25 13:50	19-07-02	0	19-07-09	5
10	0	1	19-06-07 10:45	19-06-14	0	19-06-21	0
10	0	0	19-06-07 11:40	19-06-14	10	19-06-21	10
10	1	0	19-06-07 13:05	19-06-14	0	19-06-21	0
10	1	1	19-06-07 13:40	19-06-14	0	19-06-21	0
11	0	1	19-07-03 11:40	19-07-10	0	19-07-17	0
11	0	0	19-07-03 11:20	19-07-10	0	19-07-17	0
11	1	0	19-07-03 12:50	19-07-10	0	19-07-17	1
11	1	1	19-07-03 13:15	19-07-10	0	19-07-17	10
12	0	1	19-06-27 11:00	19-07-04	0	19-07-11	0
12	0	0	19-06-27 10:15	19-07-04	0	19-07-11	0

12	1	0	19-06-27 11:45	19-07-04	0	19-07-11	0
12	1	1	19-06-27 11:30	19-07-04	0	19-07-11	2
13	0	1	19-07-11 10:15	19-07-18	10	19-07-25	10
13	0	0	19-07-11 10:40	19-07-18	0	19-07-25	0
13	1	0	19-07-11 11:10	19-07-18	0	19-07-25	0
13	1	1	19-07-11 11:30	19-07-18	0	19-07-25	1
14	0	1	19-07-09 11:20	19-07-16	0	19-07-23	0
14	0	0	19-07-09 12:20	19-07-16	0	19-07-23	0
14	1	0	19-07-09 14:20	19-07-16	0	19-07-23	0
14	1	1	19-07-09 14:40	19-07-16	10	19-07-23	10
15	0	1	19-07-17 11:20	19-07-24	10	19-07-31	10
15	0	0	19-07-17 10:40	19-07-24	0	19-07-31	0
15	1	0	19-07-17 13:40	19-07-24	0	19-07-31	0
15	1	1	19-07-17 13:15	19-07-24	1	19-07-31	1

Appendix C. Number of eggs predated per nest after 1 and 2 weeks in the nest predation-release experiment conducted between June 7th and July 31st, 2019. “Road” (Road-Adjacency = 1) treatment nests were on the road shoulder, while “No-Road” (Road-Adjacency = 0) treatment nests were in sandy or loamy soil under open canopy approximately 1 km away from the road. “Linear” (Configuration = 0) treatments had nests placed 3 m apart along a linear transect. “Clumped” (Configuration = 1) treatments had grouped nests within 2 m of their nearest neighbours. Eggs were considered predated if there were missing or destroyed.

Block	Road-Adjacency	Configuration	Predated Eggs per Nest Week 1										Predated Eggs per Nest Week 2									
			Nest										Nest									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	0	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
1	0	0	4	1	2	0	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	
1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	1	0	0	0	0	0	0	0	0	0	0	2	4	0	4	4	0	4	0	2	
2	0	1	0	0	0	0	0	0	4	0	0	0	0	0	0	0	4	4	0	4		
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
2	1	1	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	0	4		
3	0	1	0	0	0	0	0	0	0	0	0	0	0	4	0	4	4	0	0	4		
3	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4		
3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	2	0	4		
3	1	1	0	1	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4		
4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0		
4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
4	1	1	0	0	0	0	0	0	0	0	0	0	0	4	4	4	0	4	3	4		
5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
5	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	2	0	0		
5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
6	0	1	3	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	3		

6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0
7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	4	0	0
8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4
9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	4	0	4
10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
11	1	1	0	0	0	0	0	0	0	0	0	0	0	0	4	4	2	4	4	1
12	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
13	0	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

13	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
15	0	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1	1	3	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0

Appendix D. Additional estimates of the effect of (i) crop cover and (ii) forest cover within 500m on our GLMM estimating the effect of road adjacency and nest configuration on survival of artificial turtle nests. Each group of 10 nests (10 nests=site; 59 sites altogether) was either adjacent to a road or approximately 1 km from a road and in either a clumped or linear configuration. (* indicates p-value <0.05, ' indicates p-value <0.10).

(i) Crop Cover

Response Variable	Predictor Variable	Estimate	SE	z	P
Survival	Intercept	5.395	2.482	2.174	0.029*
	Road Adjacency	3.956	2.156	1.834	0.066'
	Configuration	-0.367	2.289	-0.16	0.872
	Configuration : Road Adjacency	-5.932	3.158	-1.878	0.060'
	Crop Cover	-0.039	0.026	-1.504	0.132

(ii) Forest Cover

Response Variable	Predictor Variable	Estimate	SE	z	P
Survival	Intercept	1.869	1.877	0.996	0.319
	Road Adjacency	4.179	2.101	1.989	0.046*
	Configuration	-0.337	2.196	-0.154	0.878
	Configuration : Road Adjacency	-5.085	3.031	-1.915	0.055'
	Forest Cover	0.036	0.028	1.283	0.199

Appendix E. Sensitivity analyses to estimate the range of likely values of adult road kill rate that can be compensated by the observed predation release on roadside nests. (i) Juvenile mortality estimates were either increased or decreased by 50% relative to the values estimated by Midwood et al. (2015, Table 1). (ii) Initial population size was reduced by either 50% or 90%. In each case, final population size estimates are of barely stable painted turtle populations derived from PVAs for both the natural scenario (far from roads, with a clumped nest configuration) and the predation-release scenario (adjacent to roads, with a linear nest configuration). The difference in adult mortality rate for the two populations is the amount of adult mortality compensated by reduced nest mortality in the predation-release population. Reduced nest predation compensates for 5.4% additional adult mortality when juvenile mortality is 50% lower, and 4.1% when juvenile mortality is 50% higher. Reduced nest predation compensates for 4.7% additional mortality when the initial population size is reduced by 50%, and 3.5% when the initial population is reduced by 90%.

(i) Juvenile mortality rate	Scenario	% Adult Mortality	Final Population Size (SD)
50% lower	natural	8.3	828.77 (144.19)
	predation release	13.7	832.96 (151.72)
50% higher	natural	2	865.41 (105.69)
	predation release	6.1	838.21 (136.65)

(ii) Initial population size	Scenario	% Adult Mortality	Final Population Size (SD)
Reduced by 50%	natural	5.4	381.92 (84.27)
	predation release	10.1	360.59 (112.25)
Reduced by 90%	natural	3.9	85.99 (15.28)
	predation release	7.4	86.76 (14.24)