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The impact of roadkill on the viability of three threatened Asian turtle species

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Abstract. Turtles and tortoises are one of the most endangered groups of vertebrates, especially in Asia, a hotspot for reptile biodiversity. Roadkill can pose an additional threat that may affect the long-term persistence of turtle populations. The aim of this study is to assess the viability of the populations of three threatened species of turtle, the Spur-Thighed Tortoise *Testudo graeca* (VU), the Southeast Asian Box Turtle *Cuora amboinensis* (EN) and the Elongated Tortoise *Indotestudo elongata* (CR), in five areas in Asia. We developed a spatially implicit model using demographic parameters of each species and estimated roadkill rates (ind/km/year) to predict how the populations will evolve in the next 20 years with the assumption that the observed roadkill persists. We also estimated the proportion of mortality necessary to lead these populations to local extinction. Roadkill as an additional source of mortality is unlikely to impact the studied populations. Approximately 50% of annual mortality is necessary for the populations to no longer be viable. Unexpectedly, conservation status had no significant effect on the degree to which species are impacted by roadkill. Only Southeast Asian Box Turtle populations decreased drastically, not due to roadkill, but most likely because of the low recorded adult survival rates. Future research should focus on better understanding the influence of other threats in combination with roadkill, as well as doing more population studies that consider the specific demographic parameters of local populations of turtles.

Key words. Testudines, *Cuora amboinensis, Indotestudo elongata*, mortality, risk of extinction, road mortality, *Testudo graeca,* tortoise, turtle.

Introduction

Turtle and tortoise populations have been declining at an alarming rate, with the IUCN Red List (version 2025.1) (IUCN 2025) and the IUCN Tortoise and Freshwater Turtle Specialist Group (TFTSG) Provisional Red List estimating that more than 50% of the currently recognized species are threatened (Rhodin et al. 2025). In 2017, 179 species were Vulnerable (VU), Endangered (EN) or Critically Endangered (CR) (Rhodin et al. 2017), a number that rose to 183 species in 2021 (Rhodin et al. 2021). According to a recent assessment from 2025 (Rhodin et al. 2025), 196 species are now threatened. This number keeps on rising, making turtle and tortoise among the most endangered groups of vertebrates (Rhodin et al. 2017, Stanford et al. 2020, Rhodin et al. 2021, Rhodin et al. 2025). Asia is a hotspot of reptile biodiversity with the highest proportion of threatened turtle species (Roll et al. 2017, Rhodin et al. 2018, Stanford et al. 2020), underscoring its importance as a priority region for turtle conservation.

Turtles are being driven to extinction mainly because of habitat loss and degradation, climate change, invasive species and over-harvesting for consumption, medicinal purposes and the pet trade (Stanford et al. 2020). Turtles may be particularly vulnerable to roads (Beaudry et al. 2008), as they fragment habitats (Baldwin et al. 2004) and isolate populations, making it difficult to find mates and nest sites (Andrews et al. 2015) and ultimately affecting the gene flow (Gallego-García et al. 2018). Their behavioural response to approaching vehicles is also of concern, as many species move very slowly when crossing roads or retreat into their shells and remain motionless when a vehicle approaches (Andrews et al. 2015). According to Gibbs & Shriver (2002), roadkill can pose a threat not only by reducing population abundance but also by causing sex-biased mortality, often as a result of increased female dispersal while searching for nesting sites (Aresco 2005, Andrews et al. 2015). Turtles live longer than most vertebrates (Gibbons 1987) and, consequently, have delayed maturity, which is a risk, as they can die before the first reproduction (Congdon et al. 1993). Moreover, most turtles are usually too small to be detected by drivers and deliberate vehicular impact is also reported (Andrews et al. 2015). These combined threats may jeopardize the persistence of local populations, with potentially severe consequences for rare or threatened species. Roadkill occurrences of turtle and tortoise species may represent a significant local threat by altering population structure and even morphology in areas adjacent to roads. For example, Patrick & Gibbs (2010) reported a higher proportion of females of the Common snapping turtle *Chelydra serpentina* within 500 m of ponds at lower road densities, and larger individuals of *C. serpentina* and the Eastern painted turtle *Chrysemys picta picta* within 100 m, suggesting selective pressures near roads. Similarly, Peaden et al. (2015) documented a decline in tortoise abundance with increasing proximity to roads, with more significant effects near large, high-traffic roads, underscoring the impact of roadkill on nearby populations. A recent study concluded that in only 17 years, a population of the Common snapping turtle that inhabits a wetland bisected by a highway declined severely (Piczak et al. 2019) and its population is at risk of extirpation due only to the isolated impact of roadkill. However, studies examining the effects of roads on turtles remain limited in number and geographic scope and the analysis of possible long-term effects on the persistence of populations are rare.

The Spur-Thighed Tortoise *Testudo graeca*, Southeast Asian Box Turtle *Cuora amboinensis*, and Elongated Tortoise *Indotestudo elongata* are all found in Asia and have multiple roadkill occurrences (Tok et al. 2011, Bernardo Jr. 2019, Silva et al. 2020, Purnomo et al. 2021, Rattanawanawong et al. 2022). Their main threats include habitat loss due to urbanization, and intense harvesting for illegal purposes and the pet trade (Lambert 1981, Rahman et al. 2019, Cota et al. 2020). The Spur-Thighed Tortoise is a diurnal (Stark et al. 2018) and terrestrial tortoise present mostly in various regions of Europe and Asia (Tortoise and Freshwater Turtle Specialist Group 1996). According to the IUCN, the species is classified as Vulnerable (TFTSG 1996). However, this assessment dates back to 1996, and its conservation status may be more severe than currently recognized. The Southeast Asian Box Turtle is a nocturnal (Stark et al. 2018), terrestrial and freshwater turtle distributed throughout Southeast Asia (Cota et al. 2020). It is considered Endangered (EN), and its global population is decreasing, but it is a resilient species, being able to survive in urbanized areas (Cota et al. 2020). The Elongated Tortoise is crepuscular and nocturnal (Stark et al. 2018, Rahman et al. 2019), distributed across South and Southeast Asia (Rahman et al. 2019), and currently classified as Critically Endangered (CR) due to a declining global population, driven by its inability to persist in urbanized habitats. Already rare or uncommon in most of its range, the species is expected to survive in the future only within protected areas (Rahman et al. 2019).

The aim of this study is to evaluate the viability of Spur-Thighed Tortoise, Southeast Asian Box Turtle, and Elongated Tortoise populations under increased road mortality pressure. We developed a spatially implicit model to assess how populations respond over time to observed roadkill levels and recorded life-history traits of each species, sourced from a literature review. Additionally, we identify mortality thresholds above which populations are unlikely to persist. This assessment provides critical insights for conservationists and road managers to understand the true impact of roadkill and to target mitigation efforts that support the long-term survival of these species in the affected areas.

Methods

Study area

We searched for studies from the past 15 years reporting observed roadkill data for the Spur-Thighed Tortoise, Southeast Asian Box Turtle, and Elongated Tortoise. To reduce the risk of underestimating roadkill rates, we included only studies with a survey interval of no more than seven days. We identified five study areas with roadkill records for the Elongated Tortoise (n = 1), Spur-Thighed Tortoise (n = 1), and Southeast Asian Box Turtle (n = 3) (Fig. 1).

Between 2005 and 2009, from May to June, Tok et al. (2011) recorded 26 Spur-Thighed Tortoise roadkill carcasses during surveys conducted along roads across the Anatolian region of Turkey, covering approximately 20,000 km in total. The region features a mixed landscape of forest, agricultural fields, and widely spaced cities (Fig. 1A), and has the highest road density among the study areas.

From February 2018 to January 2019, Rattanawanawong et al. (2022) analysed 48 km of the main road that crosses the Khao Yai National Park, in Thailand, starting on Highway 2090 and ending on Highway 3077. The landscape is characterized by ravines, hills, many creeks, and grasslands and marshes (Fig. 1B). In this study, two roadkills of the Elongated Tortoise were observed.

As for the case studies where roadkill of the Southeast Asian Box Turtle was reported, in the first study site, Bernardo Jr. (2019) analysed a 67 km segment of a national highway (Puerto Princesa South Road) in the Philippines, from January 2010 to December 2015, recording 102 roadkills. The landscape includes natural habitats like forests, grasslands, rivers and ponds, but also urbanized areas with agricultural fields and residential areas (Fig. 1C). In the second study (Fig. 1D), carried out from February to March 2020, Purnomo et al. (2021) observed three roadkills. The study surveyed the 22.4 km highway that crosses the Rawa Aopa Watumohai National Park in Indonesia, where savanna is the dominant ecosystem. In the third study site (Fig. 1E), Silva et al. (2020) conducted a study from 2015 to 2017 analysing a 47.8 km section of the Highway 304, which crosses the Dong Phayayen-Khao Yai (DPKY) World Heritage Site, and detected nine roadkills.

Roadkill estimation

For each study, we compiled the number of roadkill records, survey period (start and end dates), number of surveyed days, survey interval (number of days between consecutive surveys), and total road length surveyed. All analysed roads were paved. We then calculated the roadkill rate per surveyed day and per survey period, and standardised to a yearly rate, using the following formulas:

$$\text{Roadkill rate based on surveyed days} = \frac{N}{K \times S} \times 365$$

$$\text{Roadkill rate based on survey period} = \frac{N}{K \times Sp} \times 365$$

where N = number of roadkills observed for a species, K = road length in km, S = number of surveyed days, and Sp = survey period (in days). We assumed roadkill occurred at a constant rate throughout the year.

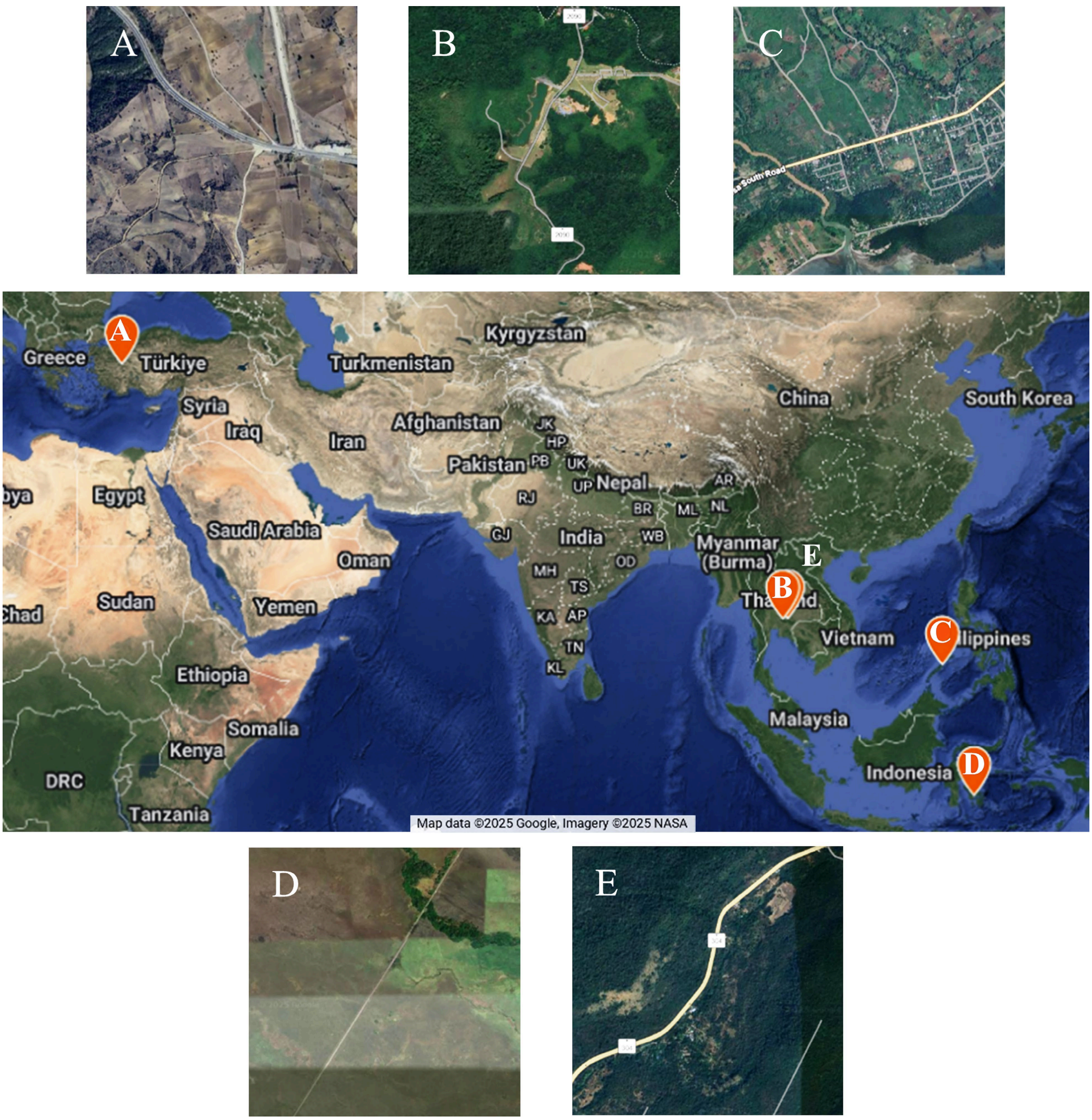

Figure 1. Five study areas (red icons): (A) Spur-Thighed Tortoise *Testudo graeca* in the Anatolia region (Turkey) (Tok et al. 2011); (B) Elongated Tortoise *Indotestudo elongata* in Thailand (Rattanawanawong et al. 2022); (C) Southeast Asian Box Turtle *Cuora amboinensis* in the Philippines (Bernardo Jr. 2019); (D) Southeast Asian Box Turtle *Cuora amboinensis* in Indonesia (Purnomo et al. 2021); (E) Southeast Asian Box Turtle *Cuora amboinensis* in Thailand (Silva et al. 2020).

Table 1. Information collected from each study with observed roadkill data (NA – not known).

Species	Southeast Asian Box Turtle			Elongated Tortoise	Spur-Thighed Tortoise
	Bernardo Jr. (2019)	Purnomo et al. (2021)	Silva et al. (2020)	Rattanawanawong et al. (2022)	Tok et al. (2011)
Country	Philippines	Indonesia	Thailand	Thailand	Turkey
Year	2010–2015	2020	2015–2017	2018-2019	2005-2009
Nº of roadkill	102	3	9	2	26
Surveyed days	288	28	100	207	17
Surveyed road length (km)	67	22.4	47.8	48	4858
Survey period (days)	2191	28	1096	365	1522
Survey interval (days)	NA	1	NA	2	NA
Roadkill rate per surveyed day	1.929	1.746	0.687	0.073	0.115
Roadkill rate per survey period	0.253	1.746	0.063	0.042	0.001

While the roadkill rate per surveyed day tends to overestimate mortality, as it extrapolates a certain value of mortality to every day of the year, the roadkill rate per survey period can underestimate the mortality, because in the period considered, surveys were not performed every day, and some carcasses may have disappeared between visits. We used both estimates for our analyses to have a range of minimum and maximum roadkill rates to generate best- and worst-case scenarios (Table 1).

We generated a 1 × 1 km grid square centred on the surveyed road in each study area and estimated the total road length within this grid cell using the geometry calculation tools in QGIS Desktop 3.40.7 (QGIS Development Team 2025) applied to the road network dataset from Meijer et al. (2018). This allowed us to estimate the total number of roadkills by multiplying the road length (RL) with the estimated rates for each study.

Demographic data collection

We gathered demographic data for the three Asian species, including: sexual maturity, clutches per year, interclutch interval, clutch size, adult survival rate, maximum longevity, home range, and population density (Table 2).

For the population density, we prioritised values recorded from sites proximate to the study areas or within the same country, given the substantial variation observed in this parameter across different regions. The minimum and average interclutch interval were calculated using the minimum and maximum values recorded for clutches per year. When species-specific demographic data were not available, we used values from congeneric species or from species with comparable ecological and life-history characteristics (Table 2).

Data analysis

We used mortalityRISK, a user-friendly application (Neves & Grilo, unpubl.), which implements an age-structured, spatially implicit stochastic model based on that of Borda-de-Água et al. (2014), to assess the impact of roadkill on turtle populations adjacent to the surveyed roads at each study site.. In the model, during each iteration, which in the current study lasts one month, an individual can die or reproduce, with birth and death probabilities being applied in a random order. This means that an individual can even give birth and die in the same step or die in the same step they are born. For each individual, the probability of surviving is (1-Death Probability) × (1-Roadkill Probability). The Death Probability is obtained from the Adult Survival Rate and randomly varies from -10% to +10% in each iteration, accounting for bad and good months with higher and lower mortality, respectively. As for the Roadkill Probability (RP), it is the proportion of roadkilled individuals based on the starting population, calculated as follows (with SP = starting population, RR = roadkill rate, and RL = road length):

$$RP = \frac{(SP - RR \times RL)}{Sp}$$

Adult females that exceeded the age of sexual maturity and that have not reproduced in Minimum Iterations between Birth (MIB), have a breeding probability calculated based on Average Iterations between Birth (AIB), if an adult male is present. Therefore, the breeding probability is calculated such that successful reproduction occurs on average once in [AIB–MIB] iterations and, assuredly, at least once in [AIB–MIB+AIB] iterations. For each breeding event, the number of offspring is randomly sampled from a uniform distribution ranging from 1 to the maximum clutch size, with each offspring having an equal probability of being male or female. Finally, if an individual exceeds the age defined by its maximum longevity, we assume it has died. In cases where the number of individuals in the simulation exceeds the maximum population threshold, random individuals are removed until the population returns to the predefined limit, thereby enforcing a density-dependent population regulation. For the simulations presented here, the maximum population was defined as twice the initial population size. This value was selected because it was sufficiently high to allow population growth or decline to be clearly detected, while remaining low enough to avoid unnecessary computational costs.

Table 2. Demographic parameters of each species (minimum, maximum and average). Supplementary material includes the demographic parameters with the respective references (Supplementary Table 1). * Value found for *Chrysemys picta* with a similar life cycle; ** Value found for *Chrysemys picta marginata* with a similar life cycle.

Species	Southeast Asian Box Turtle			Elongated Tortoise	Spur-Thighed Tortoise
	Bernardo Jr. (2019)	Purnomo et al. (2021)	Silva et al. (2020)	Rattanawanawong et al. (2022)	Tok et al. (2011)
Demographic parameters					
Population density min–max (average) (ind/ha)	3.1–9.2 (6.15)	9.9–65.78 (37.84)	0.82–65.78 (33.3)	2.25–2.43 (2.34)	1.67–21 (11.345)
Sexual maturity age min–max (average) (days)		2007.5–2190 (2098.75)		3650–5110 (4380)	4383
Clutch size min-max (nº of eggs)		1–6		1–9	1–8
Clutches per year min–max		1–3		1–2	1–4
Minimum interval between births (days)		121.667		182.5	91.25
Average interval between births (days)		243.333		273.75	228.125
Home range min-max (average) (km²)		0.001**–0.079** (0.040)		0.038–0.7 (0.369)	0.003–0.017 (0.01)
Adult survival rate max–min (average)		0.29*–1* (0.645)		0.76–1 (0.88)	0.88–0.99 (0.935)
Maximum longevity (months)		458.4		240	1392
Road mortality					
Roadkill rate min-max (average)	0.254–1.929 (1.092)	1.746–1.746 (1.746)	0.063–0.687 (0.375)	0.042–0.073 (0.058)	0.001–0.115 (0.058)

Table 3. Buffer distance defined for each species, based on their average home range values, and calculated minimum, maximum and average starting populations based on buffer distance, road length and population density. * Value calculated from the home range values of *Chrysemys picta marginata*, which has a similar life cycle.

Species	Southeast Asian Box Turtle			Elongated Tortoise	Spur-Thighed Tortoise
	Bernardo Jr. (2019)	Purnomo et al. (2021)	Silva et al. (2020)	Rattanawanawong et al. (2022)	Tok et al (2011)
Average home range (km²)	0.04*	0.04*	0.04*	0.369	0.010
Road length (km)	1.060	1.186	1.118	1.475	3.845
Buffer distance (km)	0.226	0.226	0.226	0.686	0.113
Minimum starting population (ind)	148.299	530.094	41.372	455.118	144.817
Maximum starting population (ind)	440.115	3522.179	3318.861	491.528	1821.055
Average starting population (ind)	294.207	2026.136	1680.116	473.323	982.936

Assuming a closed population, we analysed the impact of road proximity on each population at a specific distance from the road (buffer). By considering the average home range value of each species (Table 2) as a circular area, we defined buffer distances (BD) as the diameter of the corresponding circle. The total number of individuals within the buffer (SP) was estimated by multiplying species-specific population density (minimum, maximum and average) by the buffer area [SP = 2×BD×RL×Population density] (Table 3).

We ran models for each species and study area over a 20-year period, starting on January 1, 2025. Each model was simulated under two conditions: without roadkill and with roadkill, using best-case and worst-case scenarios. For the worst-case scenario, we used the lowest values for population density and the highest values for roadkill rate. The best-case scenario used the opposite parameter values. All other parameters were held constant across scenarios, including the adult survival rate and maturity age, where we used the average values.

While keeping every parameter at their average values, we also estimated the threshold of annual mortality above which the populations are no longer viable, corresponding to an abrupt decrease in survival. To determine these specific population viability thresholds, for each case, we considered the value of mortality from which less than 1% of model repetitions had an individual surviving. This allowed us to exclude outliers that would have otherwise affected the obtained mortality threshold values. This means that the population viability thresholds we obtain are the annual mortality values upon which we have 99% certainty that the population will go locally extinct in 20 years or less, under the simulated conditions.

Results

The Spur-Thighed and Elongated Tortoises had similar responses (Figs 2 and 3). Both populations grew until reaching a maximum population size, although the Elongated

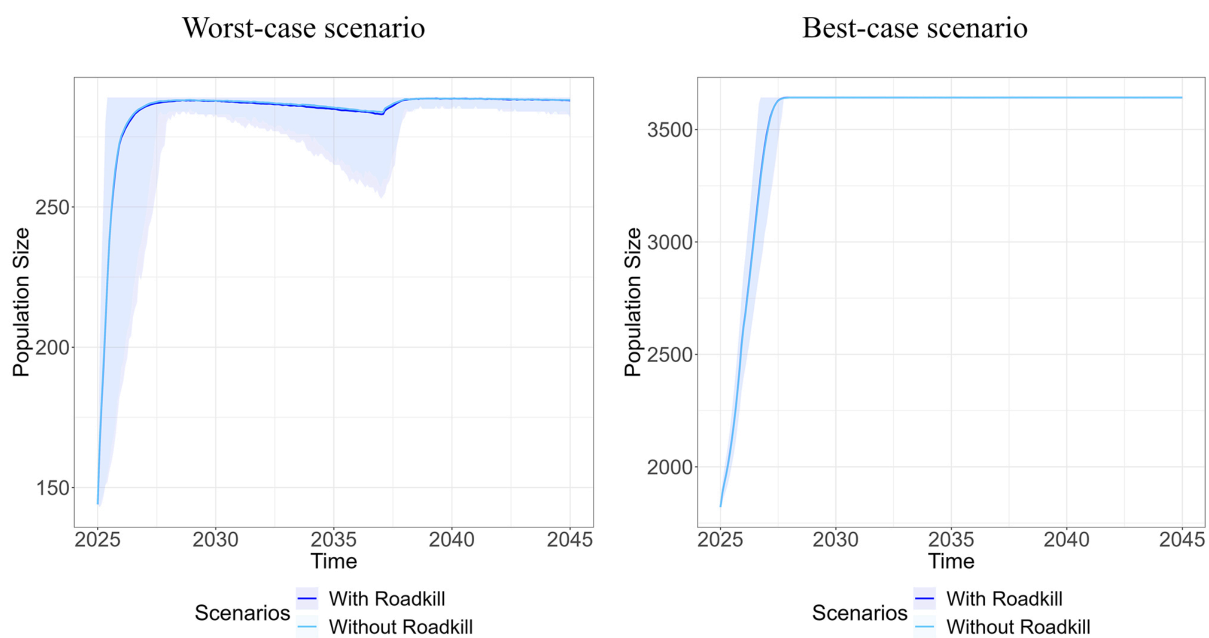

Figure 2. Evolution of the Spur-Thighed Tortoise *Testudo graeca* populations over 20 years in a worst-case scenario and best-case scenario, with and without roadkill mortality. The shaded area represents the 95% confidence interval.

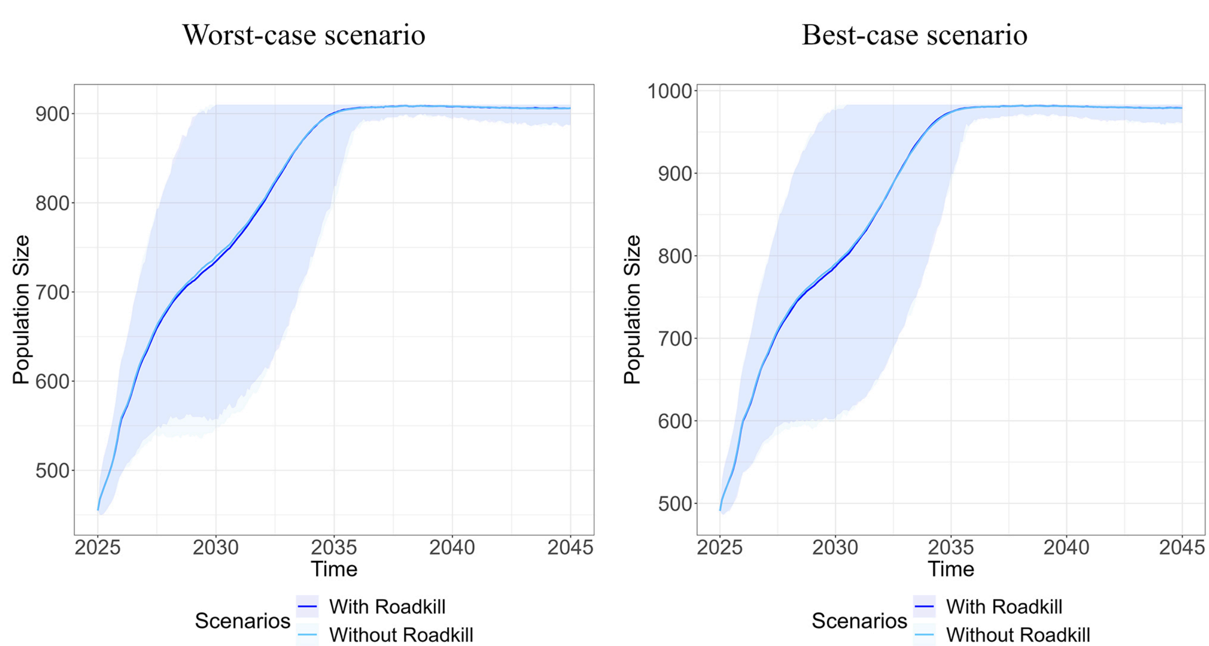

Figure 3. Evolution of the Elongated Tortoise *Indotestudo elongata* populations over 20 years in a worst-case scenario and best-case scenario, with and without roadkill mortality. The shaded area represents the 95% confidence interval.

Tortoise took more time (Fig. 3). Population trajectories were similar with or without roadkill. In all cases, populations subjected to roadkill mortality almost always showed slightly lower abundance trajectories compared to populations without road mortality. While this was also the case for the Southeast Asian Box Turtle, for these other two species, the difference was almost imperceptible.

For the Southeast Asian Box Turtle, the populations declined across all scenarios over 20 years (Figs 4–6), with several approaching near-extinction, especially in the worst-case scenario (Figs 4, 6). In the second study area, roadkill impact was negligible (Fig. 5), consistent with results observed for the Spur-Thighed and Elongated Tortoises. For the first and third study areas under worst-case

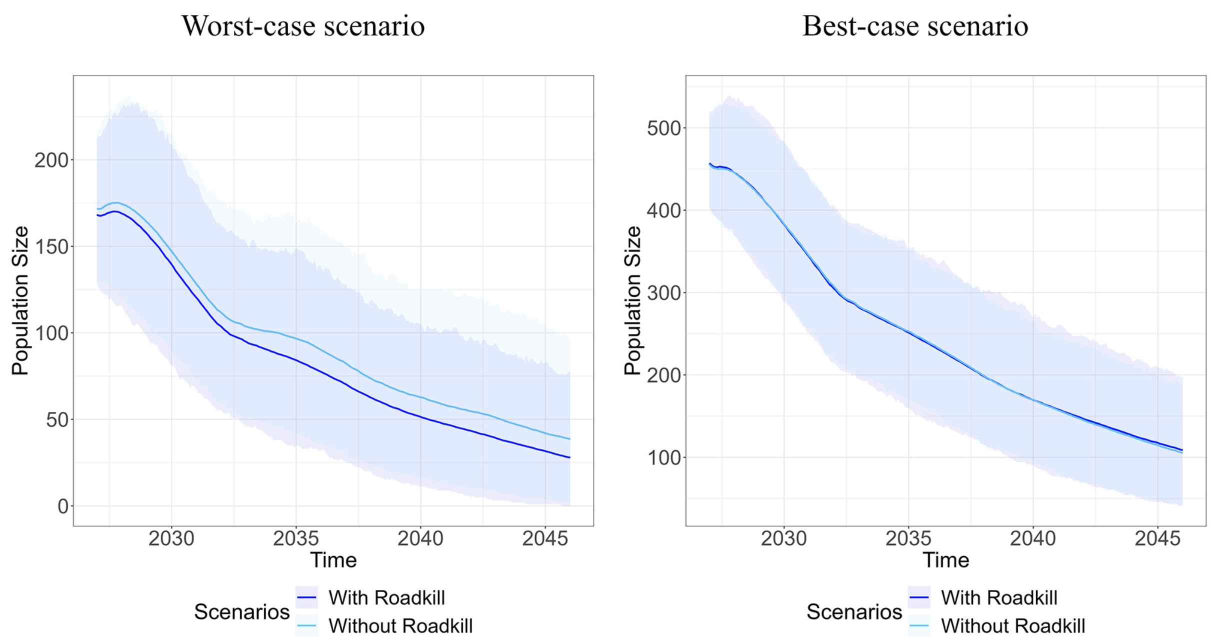

Figure 4. Evolution of the Southeast Asian Box Turtle *Cuora amboinensis* populations (Bernardo Jr. 2019) over 20 years in a worst-case scenario and best-case scenario, with and without roadkill mortality. The shaded area represents the 95% confidence interval.

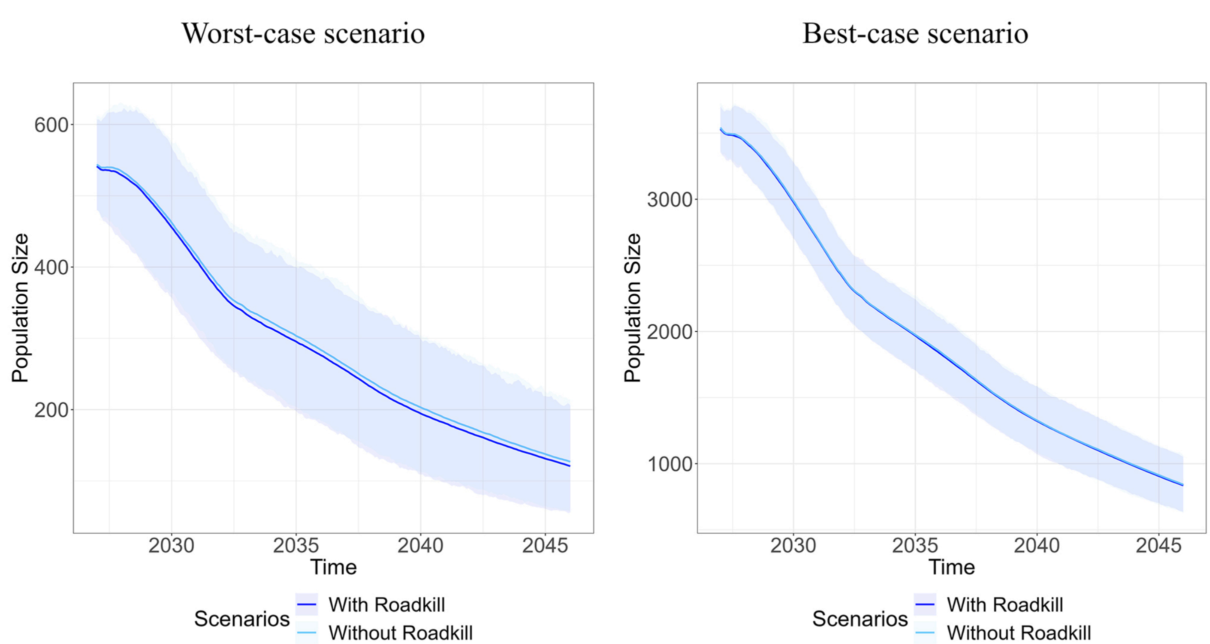

Figure 5. Evolution of the Southeast Asian Box Turtle *Cuora amboinensis* populations (Purnomo et al. 2021) over 20 years in a worst-case scenario and best-case scenario, with and without roadkill mortality. The shaded area represents the 95% confidence interval.

scenarios, there is a small difference with and without roadkill mortality. Although the probability of local extinction due to road mortality is higher, it was not pronounced (Figs 4, 6, worst-case).

Spur-Thighed Tortoise population's probability of survival started decreasing at approximately 36% annual mortality and reached the viability threshold at around 52%, for both with and without roadkill mortality (Fig. 7A). For the

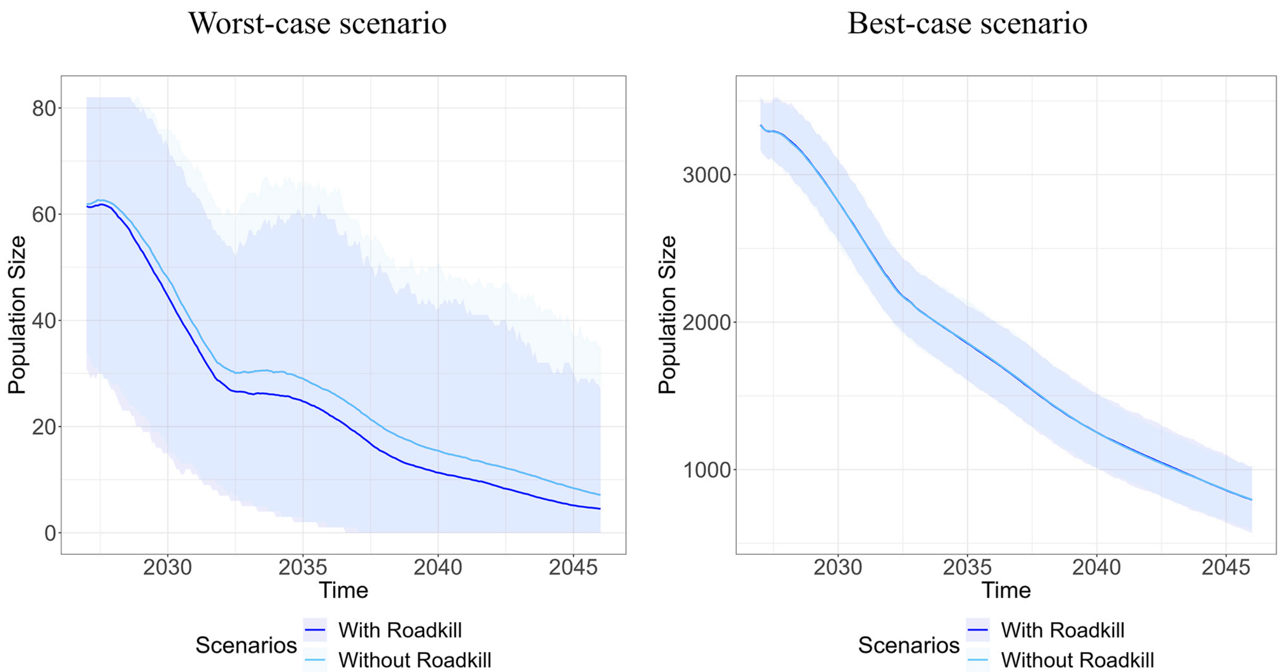

Figure 6. Evolution of the Southeast Asian Box Turtle *Cuora amboinensis* populations (Silva et al. 2020) over 20 years in a worst-case scenario and best-case scenario, with and without roadkill mortality. The shaded area represents the 95% confidence interval.

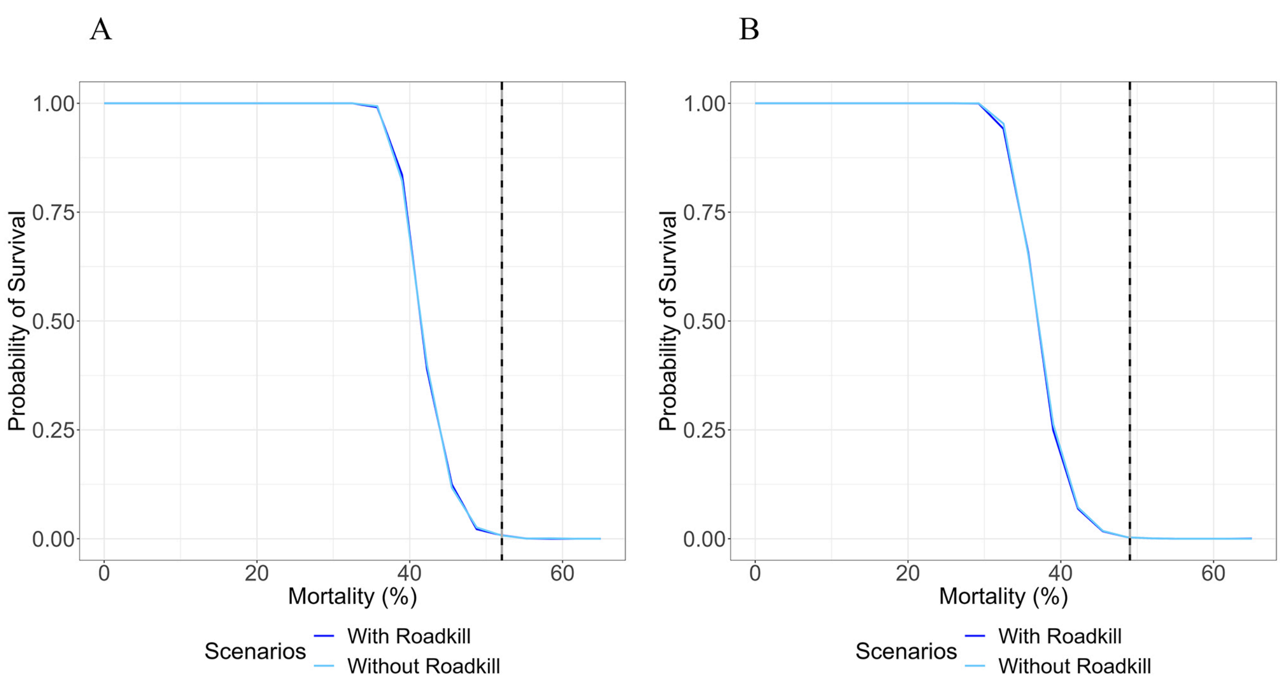

Figure 7. Threshold of mortality proportion, with and without roadkill (black and grey dashed lines, respectively), above which the populations of the Spur-Thighed Tortoise *Testudo graeca* (A) and Elongated Tortoise *Indotestudo elongata* (B) are no longer viable in each study area (Tok et al. (2011) and Rattanawanawong et al. (2022), respectively).

Elongated Tortoise, viability decline initiated at 33% without roadkill and 29% with roadkill mortality, with population failure occurring approximately at 49% annual mortality (Fig. 7B).

The Southeast Asian Box Turtle populations' probability of survival was the same with and without roadkill, and for each studied site, started decreasing at 39%, 46% and 46%, and became non-viable at approximately 55%, 59% and 59% annual mortality (Fig. 8A, 8B and 8C, respectively).

Discussion

Our results suggest that, under current mortality levels, collisions with vehicles may not be a significant driver of extinction risk for these populations. On average, populations begin to show increased extinction risk when annual removal rates reached approximately 50%.

According to IUCN assessments, roadkill is not a major threat to any of these three species (TFTSG 1996, Rahman et al. 2019, Cota et al. 2020), which aligns with our modelling results indicating that, under the specific demographic assumptions and roadkill rates considered, roadkill alone is unlikely to cause population collapse, including in worst-case scenarios with low population densities. This result probably reflects the relatively low roadkill rates across study sites. Nonetheless, roadkill rates for the Southeast Asian Box Turtle were higher than for the other two species, and a population decline associated with road mortality was observed in two out of three worst-case scenarios (Philippines and Thailand sites). In contrast, the Indonesian site showed less pronounced effects, likely due to the high population density, resulting in a larger initial population, which led to decreased roadkill relative to the total population size. Both best- and worst-case scenarios showed very similar responses in every species, regardless of roadkill impacts. Between these scenarios, the only two parameters that were not held constant were roadkill rate and population density. The population density only affects the initial and final abundances, affecting the vulnerability to roadkill also, and this difference in abundance is clearly evident when comparing the scenarios. In one scenario, the best- and worst-case roadkill rates were identical, as they were derived from the same number of survey days and identical survey durations. For the other scenarios, no appreciable variation was observed between the best- and worst-case estimates.

The Spur-Thighed and Elongated Tortoises' populations were not affected by roadkill in our model and are projected to grow over 20 years until reaching the maximum population size. The similarity of these two results can possibly be explained by their demographic parameters being either similar or compensating one another. For example, in terms of longevity, the Spur-Thighed Tortoise is the one that can live the longest and the Elongated Tortoise the shortest, but the minimum and average interval between births are the longest in the Elongated Tortoise and shortest in the Spur-Thighed Tortoise. The Southeast Asian Box Turtle falls between the two other species with regards to these parameters, thus we were expecting for its populations to respond similarly. As this did not happen, other parameters must play a role in making the populations of this species decrease while the others increase. Considering maturity age and population density parameters, the Southeast Asian Box Turtle reaches sexual maturity earlier and exhibits the highest maximum and average population densities among the three species, suggesting that it should be the least affected, particularly under the best-case scenario. However, this species also exhibits the highest absolute roadkill rates and the smallest values of maximum clutch size and minimum population density, possibly making it more prone to extirpation (Foufopoulos et al. 1999, Tian et al. 2025). Nonetheless, the key factor that might explain the severe decrease in population abundances over time is the adult minimum, and consequently average, survival rates, which are the lowest among the three species.

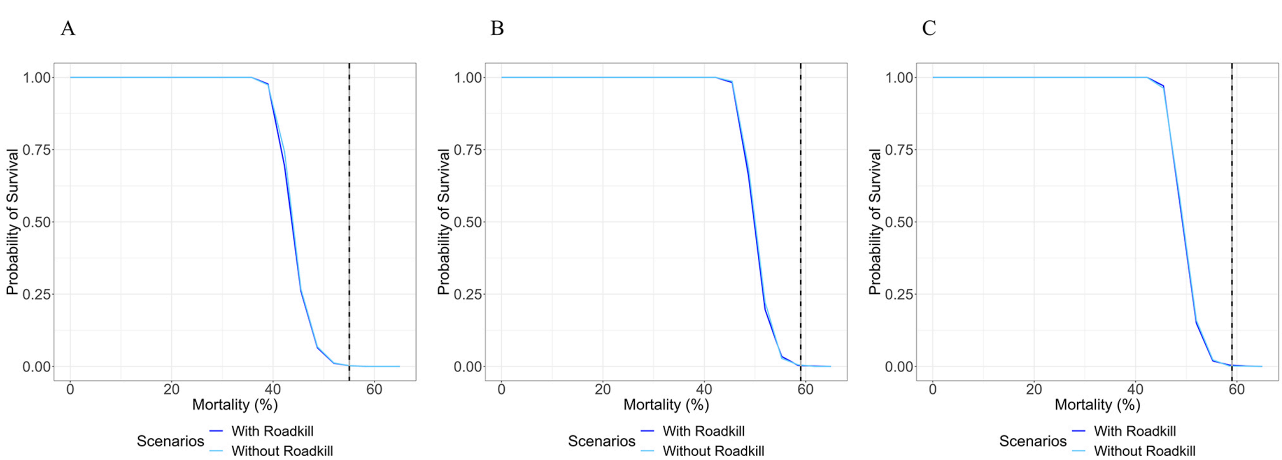

Figure 8. Threshold of mortality proportion, with and without roadkill (black and grey dashed lines, respectively), above which the populations of the Southeast Asian Box Turtle *Cuora amboinensis* are no longer viable in the three study areas (A) Bernardo Jr. (2019), B) Purnomo et al. (2021) and C) Silva et al. (2020), respectively).

Our findings show that populations' probability of survival started decreasing at an annual mortality between 29–46% and became non-viable around 50% of annual mortality (49–59%). The Elongated Tortoise started decreasing the earliest, both with and without roadkill (29% and 33%, respectively). In fact, the population of most of these threatened species is declining according to IUCN (Rahman et al. 2019, Cota et al. 2020), but for them to disappear near these roads, even with roadkill, approximately half of their populations must disappear yearly, thus showing some resilience. Although in all cases the impact of roadkill on the viability of these populations seems negligible, for the Elongated Tortoise the populations started decreasing earlier with roadkill, even if not very substantially. Despite being more vulnerable to roadkill mortality, when considering the average roadkill rates and initial abundances, the Southeast Asian Box Turtle's populations still endure a higher level of mortality. In two of its cases (Indonesia and Thailand) this could be explained by the larger abundance of the average initial populations, which results in a better resilience. Nevertheless, the Philippines population had the smallest average abundance, which should mean that the population would not sustain more mortality than the Spur-Thighed and Elongated Tortoises. However, this was not the case, as adult survival was in fact the key factor that threatens the Southeast Asian Box Turtle with local extinction. Given the same value of annual mortality, it proves to be more resilient than the other two species.

Several limitations should be considered when interpreting our results. Although turtles are usually solitary, they can form aggregations for basking, predator avoidance, and mating activities (Fenech 2023), as well as when resources or shelter are limited (Gordon & MacCulloch 1980, Feng et al. 2020). As a result, distribution can be uneven across the landscape, complicating the estimation of average population densities in extensive areas. The Southeast Asian Box Turtle, for example, is a freshwater turtle (Cota et al. 2020) and is therefore restricted to areas near waterbodies, which is not included in our model structure. Other factors, such as territoriality, interspecific competition and age- and sex-biased dispersal also require better understanding, as they can affect distribution and local abundances. Territorial behaviour, as documented in some chelonian species (e.g., *Malacochersus tornieri*; Ngwava et al. 2022), may limit local population densities and influence spatial structure. Similarly, interspecific competition through dietary overlap can affect species abundance and distribution patterns (Spencer et al. 2014), while age- and sex-biased dispersal, usually with adult males dispersing more frequently than adult females and juveniles (Sheridan et al. 2010, Fay et al. 2023), may influence the sex-ratio of a population. In the case of our model this might affect the reproductive success of the population, depending on the percentage of males and females that randomly die in our model simulations.

At the same time, there is relevant information specific to the species under study, some indicating that a few of these factors may exert a lesser influence, contributing to the validity of our results. The Spur-Thighed Tortoise may be non-territorial, considering a lack of aggression towards individuals of the same species (Burt 1943, Willemsen & Hailey 2003) and overlapping home ranges (Harless et al. 2009, Bernheim et al. 2019), while the Elongated Tortoise is sympatric with other tortoise species (Platt et al. 2010, Ihlow et al. 2016), suggesting a lack of interspecific competition. In fact, interspecific competition between tortoises is less relevant than for other reptiles and even when there are two sympatric species, they tend to partition resources, avoiding competition (Wright et al. 1988, Luiselli 2006).

In our case, roadkill did not seem to be a threat. However, estimating the relevance of these and other factors influencing species distribution and abundance, especially the major threats to chelonians such as habitat loss and illegal harvesting, is crucial, and their effects should be studied separately and combined with roadkill. We also assumed a closed population, which does not reflect natural systems. Although tortoises may exhibit site fidelity and stable home ranges at local scales (Montano et al. 2022, Ngwava et al. 2022), these data do not allow us to assume negligible immigration over the 20-year simulation horizon; therefore, our results should be interpreted primarily for closed-system or weakly connected population scenarios. Moreover, roadkill rates calculated are kept constant throughout the year, despite the influence of multiple factors. In fact, road mortality depends on conditions across the year, local characteristics, species life traits, behaviour, movement and temporal activity patterns (Ament et al. 2021, Arca-Rubio et al. 2023). Turtles can be more susceptible to roadkill when making seasonal movements in search for mates and nesting sites, or following rainfall days, for example (Beaudry et al. 2010, Cureton II & Deaton 2012, Andrews et al. 2015, Garrah et al. 2015). In some cases (Tok et al. 2011, Purnomo et al. 2021), there is a possibility of under or overestimation of mortality, since surveys were conducted over a limited period that may not fully represent the entire year. Even so, we applied the yearly average to every month, effectively minimizing the impact of this limitation and, for the Southeast Asian Box Turtle, most studies analysed are representative of the whole year, with surveys being performed in all seasons (Bernardo Jr. 2019, Silva et al. 2020). For the Elongated Tortoise, road surveys were conducted over more than 200 days with a mean interval of two days between surveys, representing a substantial monitoring effort despite the low number of recorded events. However, because roadkill rates were derived from limited sample sizes, particularly the Southeast Asian Box Turtle from Purnomo et al. (2021) and Spur-Thighed Tortoise (Tok et al. 2011), resulting estimates should be interpreted cautiously and considered exploratory. Nevertheless, we assume that surveys covering several kilometres of road can partially compensate for shorter sampling durations by increasing detection probability. Furthermore, our reference data may not reflect the characteristics of the studied populations. Demographic traits may be strongly context-dependent, highlighting the need for performing local studies in more areas to account for the specific characteristics of local populations of turtles.

For example, Willey et al. (2025) observed spatiotemporal variation in population parameters for the Spotted Turtle *Clemmys guttata*, including adult survival rate, as we have seen in the Southeast Asian Box Turtle, emphasizing the importance of long-term monitoring studies for conservation. The carrying capacity of the environment, here defined arbitrarily, is also expected to have spatiotemporal variations. However, increasing this threshold further would not alter the conclusions of the study, merely allowing populations to reach even larger sizes. Likewise, reducing the threshold would similarly have no effect on the conclusions, provided that the maximum population remained equal to or greater than the initial population size. As natural populations are unlikely to occur above the carrying capacity of the environment, we consider that the specific maximum population value used here does not meaningfully influence the results discussed. Lastly, roadkilled turtles can be difficult to detect, and even roadkill rates based on surveyed days, which overestimates road mortality events, may still not represent the real impact. Teixeira et al. (2013) estimated that reptile roadkill rates could be twice as high as observed, as carcass removal by scavengers and low detectability can lead to significant underreporting of road mortality incidents.

Conclusion

Given our model assumptions, roadkill mortality in the studied areas is unlikely to pose a significant threat to the persistence of the three threatened turtle species, regardless of scenario or conservation status. Moreover, model results suggest that approximately half of the population would need to be lost yearly before reaching non-viability thresholds, indicating a notable degree of resilience. However, the absence of site-specific demographic data and low sample sizes underscore the need for local scale research on the species life traits and roadkill data to improve the accuracy of future assessments. It is also crucial to better understand the role and relative importance of sources of anthropogenic mortality and environmental factors to identify the main drivers of turtle population dynamics. Given the low detectability of reptiles, some roadkill events are likely to go unnoticed, reinforcing the need to develop improved monitoring methods for this elusive group. Further studies are needed across broader geographic areas and ecological contexts to fully evaluate the potential impacts of roadkill.

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Supplementary data

The following data are available online:

Supplementary document 1. Demographic parameters of the studied threatened Asian turtle species with the respective references.