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Amphibian and reptile road ecology

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Published in
Frontiers in Ecology and Evolution

ISSN 1664-8714
ISBN 978-2-8325-4926-1
DOI 10.3389/978-2-8325-4926-1

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Amphibian and reptile road ecology

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Citation
Brehme, C. S., Fisher, R. N., Petrovan, S. O., Popescu, V. D., Langton, T. E. S., Andrews, K. M., eds. (2024). *Amphibian and reptile road ecology*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4926-1

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frontiers | Frontiers in Ecology and Evolution

TYPE Editorial
PUBLISHED 09 May 2024
DOI 10.3389/fevo.2024.1415003

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 09 April 2024
ACCEPTED 16 April 2024
PUBLISHED 09 May 2024

CITATION
Brehme CS, Petrovan SO, Popescu VD, Langton TES, Andrews KM and Fisher RN (2024) Editorial: Amphibian and reptile road ecology.
Front. Ecol. Evol. 12:1415003.
doi: 10.3389/fevo.2024.1415003

Editorial: Amphibian and reptile road ecology

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KEYWORDS

road, amphibian, reptile, ecopassage, tunnel, road mortality, road ecology, barrier fencing

Editorial on the Research Topic
Amphibian and reptile road ecology

Roads have complex negative impacts on biodiversity and may threaten the persistence of wildlife populations by acting as barriers to movement or sources of increased and sometimes substantial mortality (e.g., van der Ree et al., 2015; Moore et al., 2023). Amphibians and reptiles (herpetofauna) are known to be particularly susceptible to negative road impacts (e.g., Beebee, 2013; Andrews et al., 2015). Many species migrate among habitats to support basic life history requirements and must cross dangerous roads multiple times each year. Additionally, most herpetofauna species are relatively slow-moving and freeze in the presence of oncoming vehicles (Andrews and Gibbons, 2005; Mazerolle et al., 2005), resulting in an increased risk of mortality for the individual, and increased risks of decline or extirpation for vulnerable populations.

Road ecology and mitigation solutions have developed substantially in recent decades. However, progress in knowledge of their effectiveness has been hampered by a lack of post-mitigation research focused on individual and population-level responses to passage-barrier systems. Examples of critical knowledge gaps include quantified understandings of the proportions of individuals that successfully cross via passages or changes in population abundance over time (e.g., Soanes et al., 2024).

This Research Topic aimed to increase our understanding of both the effects of roads on amphibians and reptiles and the effectiveness of mitigation structures. We sought research from a diversity of regions, landscapes, and species that addressed meaningful road ecology science questions to help inform conservation. Contributed articles fell into three broad categories: 1) Characterizing road mortality and planning for mitigation; 2) Effects of roads, barriers, and passages on movement behavior; and 3) Design, efficacy, and maintenance of barriers and passages.

Road mortality hotspots are commonly used for prioritizing placement of fencing and passages; however, data available and approaches used can vary widely (Paemelaere et al., 2023; Ribeiro et al., 2023). Shin et al. compared citizen science (CS) roadkill data in the Republic of Korea to standardized published data and found advantages of widely available

CS data in increasing both geographic and temporal breadth. CS data also identified hotspots of mortality and captured observations on behavioral ecology of herpetofauna, such as temporal patterns and trends in breeding, hibernation, and habitat use. They concluded that the two types of data are complementary, and that recording spatial and temporal effort would benefit CS survey data in less-studied species. Gonçalves et al. published a standardized 6-step sampling and analytical framework for use in prioritizing mitigation actions for amphibians. The novel framework incorporates site selection, imperfect carcass persistence and detection probabilities, and higher priority values for natural areas with native cover types that are less prone to landscape transition. They then demonstrated the applicability of this approach along several roads in southern Brazil.

The probability of populations being extirpated due to road impacts may affect decisions on mitigation implementation. Wilkinson and Romansic conducted population viability analysis for California newts along a 6.6 km stretch of road with high annual mortality. Annual monitoring by citizen scientists (Parsons, 2021) coupled with a road mortality and permeability study allowed estimation of future population size in the absence of mitigation. Results predicted population extirpation in <100 years indicating a strong need for safe crossings.

Studies of species and individual movement patterns in relation to roads, barriers, and passages are paramount to informing connectivity and the design placement of these systems across the landscape. In this Research Topic, Hromada et al. recorded Mojave desert tortoise movements using GPS loggers and found that they were generally more active and made longer movements near off-highway vehicle (OHV) areas, dirt roads, and road barriers, and were less active and made shorter movements near an unfenced highway. Similarly, using accelerometers, Tipton et al. found timber rattlesnakes also made longer movements over greater time periods when encountering dirt and low-traffic paved roads relative to their movements in surrounding habitats. Both studies suggest that increased energy expenditures of reptiles near roads and barriers may be related to direct interactions to these features (e.g., avoidance, pacing back and forth) or to responses to habitat and resource modifications associated with these linear features.

Using temperature-sensitive transmitters, Sisson and Roosenburg were able to determine that timber rattlesnakes, particularly gravid females, easily breached an unmaintained barrier fence to access thermal refugia (open habitat, rock piles) available in the roadside right-of-way (ROW) habitat. In addition to fence maintenance, they suggested creating suitable thermal refugia away from the road to reduce risk of vehicle strikes and mortality from ROW maintenance. Testud et al. used PIT tags and multiple RFID antennas to monitor movements of great-crested newts within passages. They found that newts were more likely to move forward in the first meters of shorter passages, suggesting a need for research into the mechanisms responsible for this response (e.g., odor, brightness, temperature, ventilation, distance). These studies illustrate that understanding individual behavioral responses to roads, mitigation structures, and surrounding habitat may help to further understand broad-scale connectivity patterns and better inform mitigation strategies.

Once mitigation systems are constructed, it is vital to monitor their effectiveness, to verify their value, and improve future designs. Two studies in this Research Topic focus on wildlife barriers, intended to keep animals off roads and to lead them to safe passage(s). Conan et al. tested the efficacy of solid-panel permanent barriers of differing material, height, and shape (presence/absence of an overhang) with five amphibian species with different climbing and jumping abilities and in both dry and wet conditions. They found that a smooth 40-50 cm high concrete barrier equipped with a 10 cm overhang was effective in stopping the majority of amphibians. They also stressed the need for maintaining the vegetation near barriers for continued effectiveness. There is often high amphibian road mortality where barriers end (Helldin and Petrovan, 2019). Harman et al. tested the efficacy of experimental perpendicular and angled 'turnarounds' at fence ends in changing the movement trajectory of multiple amphibian species. They found that individuals of several amphibian species changed direction at the barrier turnarounds and oriented towards road passages, which supported their use for amphibian mitigation systems and corroborated their effectiveness in changing trajectories of snakes, lizards, and toads (Brehme et al., 2020). The authors cautioned that length of barrier is important, and more studies are needed to inform the design and orientation of barriers.

The permeability of under-road passages to amphibian movement can be widely variable based on biotic and abiotic passage characteristics, passage spacing, species, and location (Langton and Clevenger, 2017). In this Research Topic, enhancing the permeability of existing passages by modifying vegetation is suggested by the studies of Sisson and Roosenburg, Brehme et al., and Testud et al. also showed that enhancing permeability of passages for amphibians migrating to aquatic breeding habitats may be achieved through acoustic enrichment (playing frog calls).

Spacing passages in between long stretches of road lined with barriers can result in a large proportion of animals not finding passage entrances due to 'giving-up' (e.g., Ottburg and van der Grift, 2019; Brehme et al., 2021). Brehme et al. designed and tested a novel elevated road segment (ERS), similar to a low terrestrial bridge, that was placed on top of an existing road. The 20-cm high and 30-m long prototype was composed of road mats on top of billet support bars that were perpendicular to the road. The design negates or reduces the need for barriers as it creates open passages that are continuous across its length. Results of monitoring over four years showed this was effective for a large number of amphibian, reptile, and small mammal species and offered a new design option for crossings that can be deployed to any length.

Finally, maintenance of mitigation structures is extremely important and short-term studies may not be reflective of future effectiveness (e.g., Sisson and Roosenburg; Hedrick et al., 2019). One reason for changes in use may be due to accumulation of pollutants within passages. Over four sites across the UK, White et al. showed significant increases in a variety of chemicals in both closed-top and open-top passages over time, with most passages having elevated pH, copper, lead, and total petroleum hydrocarbon levels. As amphibians are particularly susceptible to chemical pollutants due to their permeable skin, this study highlighted important considerations for monitoring, maintenance, and design of amphibian passages.

The long-term conservation of herpetofauna requires adequate planning for habitat connectivity to facilitate movement and allow adaptation. This includes designing, installing, and maintaining safe and effective crossing structures for linear transport infrastructure. Often, when a target species is documented using a crossing, it seems natural to consider the problem solved. However, when high connectivity is needed, installation of inadequate passage-barrier systems may reduce the proportion of animals successfully crossing the road and result in population decline (e.g., Ottburg and van der Grift, 2019). In addition, passage use may increase or decrease over time, but this pattern is infrequently captured as long-term studies are rare. The studies in this Research Topic contribute to enhancing our understanding of reptile and amphibian response to roads, barriers, and passage systems, and further inform mitigation planning, design, and maintenance. Well-designed and prioritized research is needed to address the importance of passage system attributes in enhancing crossing rates, as well as long-term population monitoring of all life stages, to assess the effectiveness of these systems for maintaining viable populations.

Author contributions

CB: Writing – original draft, Writing – review & editing. SP: Writing – original draft, Writing – review & editing. VP: Writing – original draft, Writing – review & editing. TL: Writing – original draft, Writing – review & editing. KA: Writing – original draft, Writing – review & editing. RF: Writing – original draft, Writing – review & editing.

Conflict of interest

Author TL was employed by the company Herpetofauna Consultants International Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 12 August 2022
DOI 10.3389/fevo.2022.944318

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SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 15 May 2022
ACCEPTED 11 July 2022
PUBLISHED 12 August 2022

CITATION
Shin Y, Kim K, Groffen J, Woo D, Song E and Borzée A (2022) Citizen science and roadkill trends in the Korean herpetofauna: The importance of spatially biased and unstandardized data. *Front. Ecol. Evol.* 10:944318. doi: 10.3389/fevo.2022.944318

Citizen science and roadkill trends in the Korean herpetofauna: The importance of spatially biased and unstandardized data

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Roadkills are a major threat to the wildlife in human-modified landscapes. Due to their ecology, relatively small size, and slow movement, amphibians and reptiles are particularly prone to roadkill. While standardized roadkill surveys provide valuable data for regional roadkill trends, such surveys are often resource-intensive and have limited geographic coverage. Herein, we analyzed a roadkill dataset of the Korean herpetofauna derived from the citizen science database iNaturalist and compared the overall roadkill trends detected in the iNaturalist data with standardized survey-based literature data. Our results show that the overall citizen science data provide a good picture of roadkill trends for the Korean herpetofauna in terms of recorded species. We detected both similarities and notable differences between the iNaturalist and literature data. The most notable differences between the two datasets were found in the number of recorded species, distribution across habitat types, and distribution across elevational ranges. Even with spatially biased sampling, the iNaturalist data had a considerably broader geographic coverage compared to standardized surveys. In addition, we related the presence of roadkills of amphibians and reptiles to the presence of agricultural lands, forests, and grassland. While the unstandardized nature of the citizen science data can be criticized, we argue that this feature also acts as an advantage for this type of data, as citizen science can better detect roadkills of rare species or seasonal events, such as mass migration of amphibians, and inform population trends and threats. Thus, our results highlight the importance of spatially biased and unstandardized citizen science data for roadkill detection. This study builds on previous studies demonstrating citizen science as a viable method of roadkill surveys.

KEYWORDS

citizen science, conservation, herpetofauna, Republic of Korea, wildlife vehicle collisions

Introduction

Anthropogenic alterations of the landscapes result in numerous negative pressures on wildlife (Swaisgood, 2020). Among these stresses are urbanization and the associated increase in road density and traffic volume (Vercayie and Herremans, 2015). Roads tend to promote human activity (Yue et al., 2019) and they have a negative impact on populations from their construction to their daily use (Trombulak and Frissell, 2000). After the harvesting of animals, roads are the largest source of anthropogenic mortality worldwide for vertebrates (Hill et al., 2019). In addition to mortality due to wildlife-vehicle collisions (hereafter roadkill), roads also result in reduced animal movement and dispersal (Forman, 1998; Forman and Alexander, 1998; Holderegger and Di Giulio, 2010), reduced population connectivity altering ecosystem dynamics (Benítez-López et al., 2010), habitat loss (Forman, 2005), and habitat fragmentation (Delgado et al., 2007).

While roadkills present challenges for conservation (Swaisgood, 2020), they can be used as an effective tool to monitor population trends, behavioral ecology, range delineation of native and invasive species, or contaminants and pathogens (reviewed by Schwartz et al., 2020). However, roadkills do not affect all wildlife equally, and some taxa are more prone to being killed (Fahrig and Rytwinski, 2009). Reptiles and amphibians are among the species most threatened by roadkills because they are generally small-bodied and relatively slow-moving (Glista et al., 2007; Andrews et al., 2015). Furthermore, their ecology increases the susceptibility to roadkills, as amphibians migrate long distances to and from breeding sites and take advantage of linear unobstructed structures, while snakes and other reptiles utilize warm road surfaces for thermoregulation and foraging (e.g., Glista et al., 2007; Colino-Rabanal and Lizana, 2012; Mccardle and Fontenot, 2016).

In this study, we focused on the overall roadkill trends for the terrestrial herpetofauna of the Republic of Korea (R Korea hereafter). The R Korea is heavily populated and anthropogenic modifications of the landscapes are widespread (Lee and Miller-Rushing, 2014; Borzée et al., 2019b), including road construction (Andersen and Jang, 2021). Currently, there is a total of 112,977 km of roads in R Korea (KOSIS, 2020), posing a significant conservation concern for the Korean wildlife (Byun et al., 2016; Choi, 2016; Kim et al., 2019, 2021a,b; Andersen and Jang, 2021). While the Korean Expressway Corporation records mammal roadkills on expressways in R Korea (36.863 between 2004 and 2019; Kim et al., 2021b), little attention has been given to the roadkills affecting the herpetofauna. The terrestrial herpetofauna of R Korea is composed of 23 amphibian (14 frog species and 10 salamander species) and 27 reptile species (12 species of snakes, six species of lizards, and 10 species of freshwater turtles). Among the 23 amphibian species, 12 are endemic to the Korean Peninsula and one is invasive (the American Bullfrog, *Lithobates catesbeianus*). In addition, 11 species are either currently listed as nationally threatened or have been identified as threatened by previous studies (Borzée et al., 2020, 2022; Borzée and Min, 2021), and two are listed as globally threatened (NIBR, 2019; https://www.iucnredlist.org/; accessed October 4, 2021). Among reptiles, none is endemic to the Korean Peninsula, eight are invasive (freshwater turtles; Lee et al., 2016), five are listed as nationally threatened, and one is listed as globally threatened (NIBR, 2019; https://www.iucnredlist.org/; accessed October 4, 2021). On the whole, the terrestrial herpetofauna of R Korea represents a relatively high endemicity (22% of the total species diversity) with many threatened species under intense anthropogenic pressure. Therefore, identifying the various threats to the Korean herpetofauna, including roadkills, is the first important step for an effective conservation planning.

Previous studies on the herpetofauna roadkills in R Korea have used standardized roadkill surveys within relatively small geographic areas (Won et al., 2005; Yang et al., 2006; Song et al., 2009; Min and Han, 2010; Kim et al., 2018b). While such studies provide valuable insights into the regional roadkill trends, they are time-consuming and resource-intensive, consequently restricted to focal taxonomic groups and geographic areas, while the threats to the herpetofauna continue to crop up (Lee and Miller-Rushing, 2014; Macias et al., 2021). However, roadkill surveys do not need to be conducted solely by professional herpetologists (Englefield et al., 2020), and the role of citizen scientists in helping herpetological research is increasingly being recognized (Borzée et al., 2019a; Gardner et al., 2019; Rowley et al., 2019). In this regard, incorporating data from citizen science programs has significant advantages when taxonomically and geographically sampling the herpetofauna roadkills. Furthermore, citizen science initiatives are continuous, not restricted by the resources constraining research, and not geographically bound. The data is therefore generated simultaneously across multiple habitat types by multiple contributors and generally this approach results in much larger datasets (Lintott et al., 2010). Detecting herpetofauna roadkills is strongly affected by spatiotemporal factors (Vercayie and Herremans, 2015), even when surveys are specifically focused on herpetofauna roadkills. Therefore, the ability of citizen science programs to record roadkills without the constraints of resources and geographic extent provides significant advantages over conventional and standardized roadkill surveys. Indeed, previous studies have shown promising benefits of utilizing citizen science programs to detect herpetofauna roadkills (Heigl et al., 2017; Chyn et al., 2019). The utility and characteristics of citizens science-based roadkill data however have not been investigated for the Korean terrestrial herpetofauna.

Therefore, our study has multiple objectives. First, we wanted to determine and highlight the threats caused by roadkills to the Korean terrestrial herpetofauna, in terms of species threatened, geographic extent, landscape characteristics, and elevation. We then aimed to determine the importance of roadkill data points from citizens science platforms, published literature, and national databases to assess the overall herpetofauna roadkill trends in R Korea and the possible use for other studies. We also compared the monthly roadkill trend for each species to their ecology, thus highlighting the ability of citizen science data to detect key ecological characteristics of the Korean herpetofauna. Therefore, our study aims to highlight the utility and benefits of citizen science in detecting the broad trends of roadkills threatening the Korean terrestrial herpetofauna.

Materials and methods

Citizen science data

In order to rapidly collect previously uploaded roadkill observations, we created a project in iNaturalist titled "Herpetofauna Roadkill in the Republic of Korea" (https://www.inaturalist.org/projects/herpetofauna-roadkill-in-the-republic-of-korea). By generating a project with specific criteria for inclusion, the observations of dead reptiles and amphibians were automatically flagged and included in the project, if fitting the criteria. The observations were included in the project only if they had photographic records, were annotated as dead animals, and were recorded from within R Korea. From the dataset thus generated, we manually removed the observations of dead reptiles and amphibians that were not roadkills, and also removed duplicate observations of the same animal made by two or more observers. For these processes, we visually inspected each photograph uploaded with the observation. We also conducted species-by-species searches for unflagged roadkill observations and we manually included them to the project database by annotating them as dead animals. We used the datapoints that were observed between 2 October 2005 to 13 August 2021. We organized the dataset by species name, year, month of observation, observer, GPS coordinates, data quality grade (either Research Grade or Needs ID, a data validation process embedded in the platform, https://www.inaturalist.org/pages/help), and Class- and Order-level classifications (Supplementary Data 1). The project we created does not have any standardized survey protocols, and therefore the data generated from the project can be regarded as opportunistically collected.

Notes on taxonomic treatment of citizen science data

In this study, we used the list of Korean herpetofauna provided by the Korean Society of Herpetologists (http://www.krsh.co.kr/html/sub0201.html) as a reference taxonomic framework. To this list we added the three recently described *Hynobius* species (Borzée and Min, 2021) and *Bufo sachalinensis* (reassigned from *B. gargarizans* for population on the Korean Peninsula; Othman et al., 2022), but we did not include the newly described *Onychodactylus* species (Borzée et al., 2022). However, the taxonomy used herein differs slightly from that used by iNaturalist. Most notably, iNaturalist recognizes a population of *Gloydius ussuriensis* on Jeju Island as a different species "*Gloydius tsushimaensis*". Although *G. tsushimaensis* is a valid taxon originally described from the Tsushima Island in Japan (Isogawa et al., 1994), there is currently no published study to support the presence of *G. tsushimaensis* on Jeju Island. Thus, we consider occurrences of "*G. tsushimaensis*" from Jeju as *G. ussuriensis* in this study, following previous studies (Song, 2007; Lee et al., 2012; Jang et al., 2016; Shin and Borzée, 2020). Similarly, while iNaturalist recognizes the presence of both *Elaphe schrenckii* and *Elaphe anomala* in the Korean Peninsula, we only recognize the former name based on previous studies using genetic and morphological data (An et al., 2010; Lee, 2011). Thus, we included the occurrences of *E. anomala* from R Korea to that of *E. schrenckii*.

Literature data

To generate an independent dataset to compare against the iNaturalist data, we utilized the roadkill records of Korean herpetofauna reported in the literature. We searched for the relevant literature by using the Environment Digital Library Search service provided by the Korean Ministry of Environment (https://library.me.go.kr/#/), Google Scholar (https://scholar.google.com/), Research Information Sharing Service (http://www.riss.kr/index.do), and the library search engine of the Kangwon National University (https://library.kangwon.ac.kr/#/). We used the following combinations of keywords (both in Korean and English): roadkill Korea, reptile roadkill Korea, amphibian roadkill Korea, herpetofauna roadkill, and wildlife roadkill. We accessed the search engines between 24 August 2021 and 5 October 2021. The search resulted in five peer-reviewed research articles, four governmental research reports, one conference proceeding, and one dissertation containing information on herpetofauna roadkills. From each publication, we extracted the frequency of roadkill detection per reported species. We then pooled the number of records for each species to generate a literature-based dataset. We compared the species-level diversity of terrestrial herpetofauna roadkills in this dataset with that of the iNaturalist dataset. The list of literature used to generate the literature dataset is shown in Supplementary Table 1. We did not include a study describing mass movement events and associated roadkills in *Onychodactylus koreanus* (Shin et al., 2020), as the observations described in that study were uploaded to iNaturalist prior to its publication. Thus, the observations described therein were included as a part of the iNaturalist data.

Statistical analyses on the citizen science dataset

As the iNaturalist observations were not normally distributed, we primarily used chi-square tests to investigate monthly trends in the number of recorded roadkills. We focused on monthly roadkill trends as many reptile and amphibian species show highly seasonal movement patterns (Shepard et al., 2008; Garriga et al., 2017), and since previous studies suggest strong monthly patterns associated with the recorded number of herpetofauna roadkills in R Korea (Song et al., 2009; Park et al., 2017). We analyzed the monthly data at different levels. First, we conducted chi-square tests for each species of amphibians and reptiles and plotted monthly roadkill trends for each species. We then superimposed the known key life-history aspects (e.g., hibernation and breeding seasons) over the monthly roadkill trends to investigate the ability of citizen science-based roadkill trends in capturing ecological signals associated with species life histories. For information on life histories, we primarily consulted the works of Lee et al. (2012) for reptiles, and Lee and Park (2016) for amphibians. For two species of snakes (*Sibynophis chinensis* and *Gloydius intermedius*) with limited life histories documented in Lee et al. (2012), we supplemented relevant information derived from recently published studies (Do et al., 2017; Koo et al., 2018; Banjade et al., 2020).

Next, as some species had small sample sizes ($n \leq 5$), we used ecological groupings to further analyze monthly roadkill patterns. For amphibians, this grouping was based on breeding ecology (spring and summer breeders), as seasonal migration to and from breeding sites is a major aspect of amphibian ecology, and it is also associated with high road mortality (Hels and Buchwald, 2001). Using breeding ecology to group species is further justified because the hibernation period (another major factor of seasonal migration) is generally similar among Korean amphibians, spanning from October to March (Lee and Park, 2016). We excluded species with highly specialized (e.g., *Bufo stejnegeri*; Lee and Park, 2016) or poorly known (e.g., *O. koreanus*; Lee and Park, 2016; Shin et al., 2020) breeding ecologies that could not be assigned to either of the two groups. Thus, we conducted separate chi-square tests on spring and summer breeders to investigate the seasonal patterns of roadkill occurrences. The group assignment based on the breeding ecology for each amphibian species is based on the study by Lee and Park (2016) and is provided in Supplementary Table 2. Regarding reptiles, we further analyzed the monthly trends at the Order level (Squamata and Testudines) and by taxonomic groups (snake, lizards, and turtles), using these groupings as a general ecological proxy. We then analyzed the monthly trends at the Class level for both amphibians and reptiles (Amphibia and Reptilia).

In addition, we plotted the monthly roadkill observations against the total monthly observations of reptiles and amphibians. We conducted this procedure separately for amphibians and reptiles to visualize whether the temporal bias of the total herpetofauna observations was also reflected in the roadkill observations. We matched the time period of total observations to that of the roadkill observations to ensure comparability of data. Finally, we assessed the overall yearly sampling efforts of the iNaturalist dataset through years 2005 to 2021, expressed as the number of observations divided by the number of participants (*sensu* Chyn et al., 2019). We performed these biostatistical analyses in R version 4.1.0 (R Core Team, 2021), implemented in the RStudio environment.

Spatial analyses

To visualize the spatial sampling intensity of iNaturalist roadkill observations, we generated kernel density maps separately for reptiles and amphibians, using the *MASS* package (Venables and Ripley, 2002) in R version 4.1.0 (R Core Team, 2021). We also visualized the spatial sampling intensity of roadkill records based on the literature data. However, we were unable to employ kernel density analysis on the literature dataset as the exact GPS coordinates of roadkill occurrence points were not provided. Thus, we extracted information on the number of provinces surveyed in each publication, thereby generating provincial "counts". We then summed up these counts for each province to obtain the "total provincial count," which we used as a literature-based representation of spatial sampling bias broadly comparable to that of the iNaturalist data (Supplementary Data 2).

Next, we investigated the elevational distribution of iNaturalist roadkill records by using the 30-arc-s resolution (∼1 km) elevation raster downloaded from WorldClim 2.1 (https://www.worldclim.org/data/worldclim21.html; Fick and Hijmans, 2017) as the template. Using the *raster* package (Hijmans, 2019) in R, we extracted elevation values from the elevation raster for each occurrence point of both amphibian and reptile roadkill records. We then categorized the obtained elevation values into range classes divided by 100 m interval, resulting in 10 classes for amphibians (spanning 0–1,241 m asl) and nine classes for reptiles (spanning 0–810 m asl). We conducted chi-square tests on the obtained elevation ranges, separately for the amphibian and reptile datasets, to test for differences in the elevational distribution of roadkill records.

We also investigated the distribution of roadkill records across different habitat types. To do so, we first obtained the 30-m resolution broad-scale land cover raster of R Korea provided by the Ministry of Environment (https://egis.me.go.kr/main.do). This land cover map is classified into seven discrete habitat categories such as, residential, agriculture, forest, grassland, wetland, barren ground, and water bodies (http://egis.me.go.kr/map/map.do?type=land). We extracted habitat types from the land cover map for each occurrence point of amphibian and reptile roadkills using the *raster* package (Hijmans and Etten, 2012) in R, and we categorized the habitat types according to the classification code of the land cover map. We then conducted separate chi-square tests for amphibian and reptile datasets to test for differences in the distribution of roadkill records across different habitat types.

For comparative purposes, we extracted information on elevational and habitat distribution of roadkill records reported in the literature (see section Literature data for the methods of literature search). To generate literature-based elevation and habitat type datasets, we first excluded four studies from the initial set of 11 studies because they either did not provide elevational and/or habitat information or the provided information was too coarse to make comparisons with the iNaturalist data (Table 1). Thus, the literature-based elevation dataset was generated from two studies for amphibians, and from three studies for reptiles (Table 1 and Supplementary Data 3), and the literature-based habitat dataset for amphibians was generated from four studies, and that of reptiles was generated from five studies (Table 1 and Supplementary Data 4). We categorized elevation data extracted from the literature in the same way as the iNaturalist data, with different upper elevation limits (due to differences in data). However, we could not apply the same habitat type categories to the literature-based data, mainly because the method of categorization used in the literature was different from our GIS-based approach. Our approach can only identify one habitat type falling in the same 30-m raster grid as our roadkill occurrence data, describing the general habitat settings of each roadkill record, whereas two habitat types immediately adjacent to the roadkill observation (with road expressed as an intersection of two habitat types) are usually recorded in the literature (Song et al., 2009; Park et al., 2017). Therefore, we categorized the habitat type of the literature-based data into the following nine classes, utilizing the classification scheme most frequently used in the literature: agriculture-agriculture, agriculture-residential, agriculture-water, forest-agriculture, forest-forest, forest-water, grassland-forest, residential-residential, and water-residential. Next, we conducted chi-square tests separately on literature-based amphibian and reptile datasets to test for differences in the distribution of roadkill records across elevational ranges and habitat types.

For further comparisons on the spatial aspects of the roadkill occurrences, we utilized another independent dataset generated by the National Institute of Ecology (NIE) in R Korea. The NIE dataset is based on the Korea Roadkill Observation System (KROS) launched in 2018 by the Ministry of Environment and the Ministry of Land, Infrastructure, and Transport (Kim et al., 2019). The roadkill data were recorded between March 2019 and September 2021. The resulting dataset is comprised of GPS coordinates and species-level identification for each roadkill occurrence point. After removing the entries with missing species-level identification, we used the same methods applied to the iNaturalist dataset to categorize elevational ranges and habitat types of the NIE data, separately for amphibians and reptiles. Next, we conducted chi-square tests separately for amphibian and reptile datasets to test for differences in the distribution of NIE roadkill records across habitat types and elevational ranges. As the NIE dataset had a considerably shorter time span compared to both the iNaturalist and literature datasets, we restricted the use of this dataset to the broad-scale comparisons of landscape and elevational aspects only.

TABLE 1 The list of published literature used to generate literature-based datasets.

References	Amphibian		Reptile	
	Elevation	Habitat	Elevation	Habitat
Cho, 2011	-	√	-	√
Kim et al., 2017b	-	-	-	-
Kim et al., 2018b	-	-	√	√
KNPRI, 2012	-	-	-	-
Koo et al., 2018	√	√	-	-
Min and Han, 2010	-	-	-	√
MOE, 2006	-	√	-	√
Park et al., 2017	-	-	√	√
Song et al., 2009	-	-	-	-
Won et al., 2005	-	-	-	-
Yang et al., 2006	√	-	√	-

Check marks (√) indicate studies used to extract environmental information, whereas dashes (-) indicate studies excluded from data analyses due to the lack of relevant information or because the coarseness of data did not permit further analyses. Some references could not be used in any of the analyses, but the citations are retained here to show that they were not ignored.

In addition, we measured the Euclidean distance between each roadkill point and seven types of land cover: residential area, agricultural land, forest, grassland, wetland, barren ground, and water bodies. This method highlights spatial patterns of roadkill and makes comparisons between land cover types more convenient (Kim et al., 2019). This analysis was conducted in QGIS 3.4.5 with the land cover map provided by the Ministry of Environment (http://egis.me.go.kr/). Prior to the statistical analyses, we excluded datapoints that were more than 5,000 m away from the focal feature. As a result, we analyzed 361 data points for amphibians and 273 points for reptiles. Then, we analyzed the data between and within classes to determine the difference in roadkill occurrence in relation to the landscape type. As the data were not normally distributed (Kolmogorov–Smirnov test, $K = 0.21$, df $= 3{,}490$, $p < 0.001$), we used a Bayesian inference for a linear regression to test for the difference between class, with distance as dependent variables

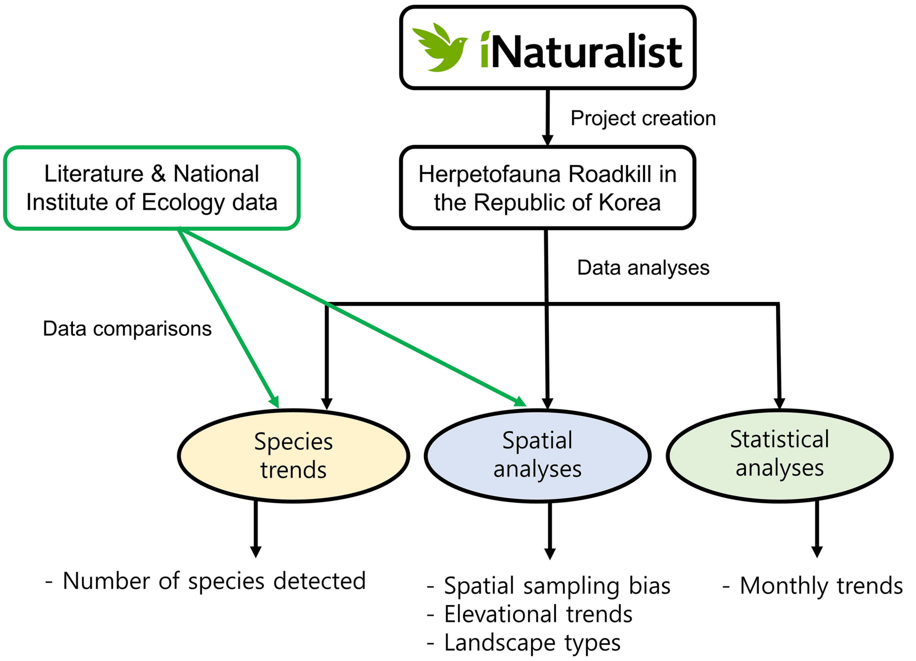

FIGURE 1
Flow chart of data analyses on the roadkill dataset collected through iNaturalist. The project can be retrieved at: https://www.inaturalist.org/projects/herpetofauna-roadkill-in-the-republic-of-korea.

and landscape class and species as independent variables. This analysis was conducted in SPSS v.28.0 (SPSS Inc., Chicago, USA). The steps of data analyses are briefly summarized in Figure 1.

Results

Citizen science data

A total of 638 observations were compiled into the project, spanning the years from 2005 to 2021 (Figure 2 and Table 2). Of the 638 recorded observations, 588 records were of research grade quality and 50 records needed further identification to be listed as Research Grade. Records in the latter category generally consisted of highly damaged *Rana* and snake roadkills, making confident identifications at the species level difficult. A total of 634 observations were retained after removing redundant observations and non-roadkill observations of deceased reptiles and amphibians (reptile $n = 273$; amphibian $n = 361$). Regarding reptiles, the iNaturalist project detected roadkills in all 11 species of terrestrial snakes native to R Korea, in 3 out of 6 lizard species (*Takydromus amurensis*, *Takydromus wolteri*, and *Scincella vandenburghi*), and in all native freshwater turtle species (*Mauremys reevesii* and *Pelodiscus maackii*). For amphibians, the project detected roadkills in all anuran species (including the invasive *L. catesbeianus*) with the exception of a recently described hylid *Dryophytes flaviventris* (Borzée et al., 2020; but refer to Section Discussion for a personal observation of roadkills in the species), and detected roadkills in two out of 10 salamander species (*O. koreanus* and *Hynobius leechii*). In total, the iNaturalist project observations detected roadkills in 76.2% of the terrestrial herpetofaunal diversity of R Korea (32 out of 42 described species). The observations also detected roadkills in several nationally endangered and near threatened species. These species include the nationally endangered Chinese Many-toothed Snake (*Sibynophis chinensis*; EN; $n = 3$), the Suweon Treefrog (*Dryophytes suweonensis*; EN; $n = 2$), the Russian Ratsnake (*Elaphe schrenckii*; VU; $n = 10$), the Gold-spotted Pond Frog (*Pelophylax chosenicus*; VU; $n = 2$), the Boreal Digging Frog (*Kaloula borealis*; VU; $n = 6$), the Reeves' Turtle (*M. reevesii*; VU; $n = 1$), and the Amur Softshell Turtle (*P. maackii*; VU; $n = 1$) (NIBR, 2019). Moreover, the project detected a roadkill of the Slender Racer (*Orientocoluber spinalis*; NT; $n = 1$), a species that is rarely and infrequently observed in R Korea (NIBR, 2019; Macias et al., 2021).

Literature data

The literature data encompassed 10,618 observations spanning years from 2003 to 2017 (Supplementary Table 1). Regarding reptiles, the literature had recorded roadkills in 10 out

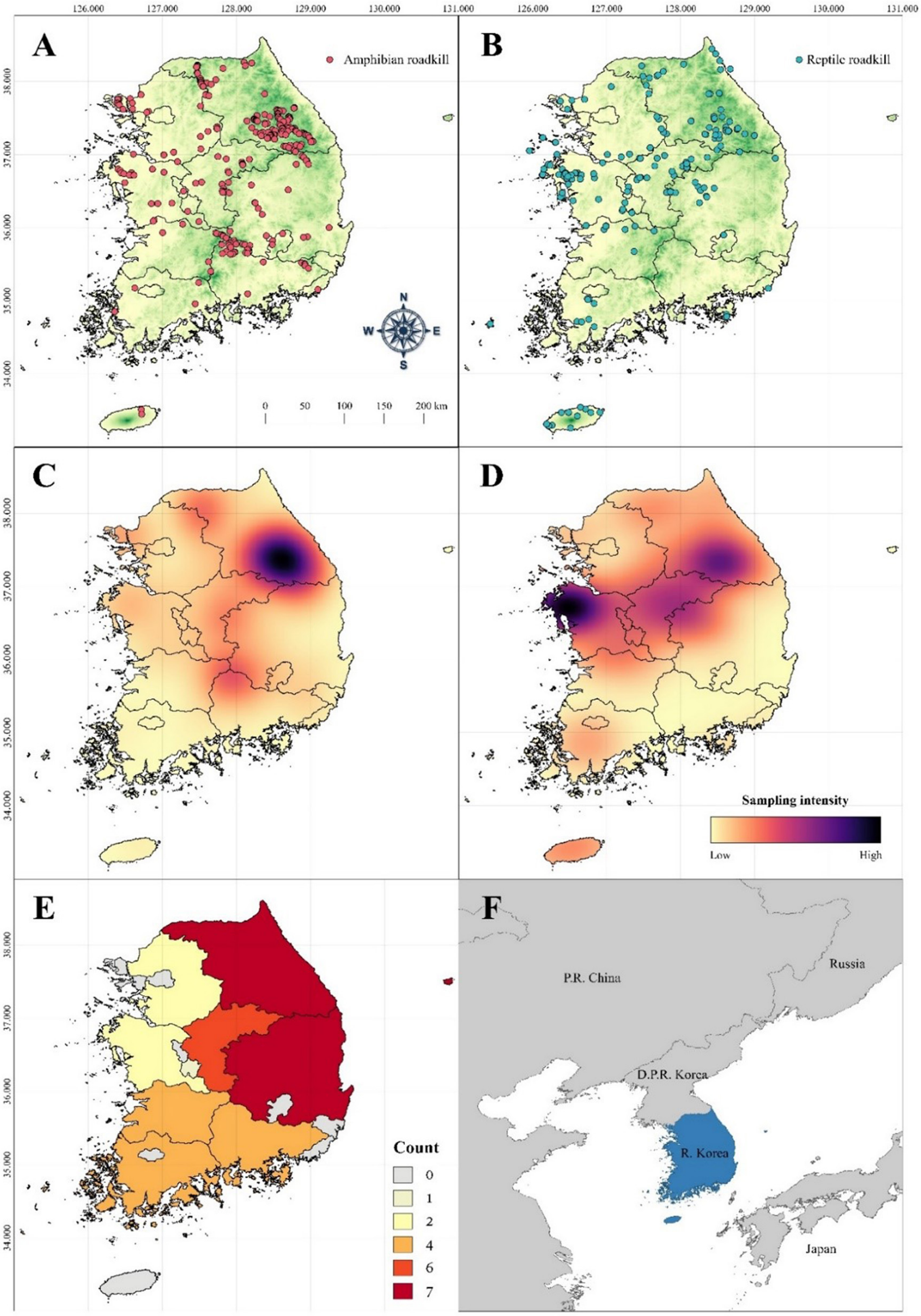

FIGURE 2
Spatial distributions of herpetofauna roadkill observations across the Republic of Korea (R Korea). **(A)** GPS coordinates of the iNaturalist amphibian roadkill observations. **(B)** GPS coordinates of the iNaturalist reptile roadkill observations. **(C)** A kernel density map of the iNaturalist

(Continued)

FIGURE 2
amphibian roadkill observations showing the spatial sampling intensity across R Korea. **(D)** A kernel density map of the iNaturalist reptile roadkill observations showing the spatial sampling intensity across R Korea. The spatial bias of roadkill observations is apparent from the cluster of records in the northern and western portions and the relative paucity of observations in the southeastern region of R Korea. **(E)** Spatial bias of the regional representation of herpetofauna roadkills based on the available literature. The unit of spatial bias (count) is a simple count of the general region surveyed (refer to Section Materials and methods for details). **(F)** The location of the study area (R Korea) in northeast Asia highlighted in blue. The map was generated in QGIS 3 with the elevation raster downloaded from the WorldClim database (Fick and Hijmans, 2017) as a base map for **(A,B)**. Darker coloration in the base map (green shade) indicates higher elevation, whereas lighter coloration (yellow shade) indicates lower elevation.

TABLE 2 **The list of Korean herpetofauna detected in the raw iNaturalist roadkill dataset ($n = 638$) at the point of data analyses, shown alongside Order-level classification, national threat status based on NIBR (2019), and the global threat status based on the IUCN Red List of Threatened Species (https://www.iucnredlist.org/; accessed 4 October 2021), if applicable.**

Species name	Order	National status	IUCN status	Number of observations
Rhabdophis tigrinus	Squamata	LC	n/a	73
Rana uenoi	Anura	LC	n/a	62
Pelophylax nigromaculatus	Anura	LC	NT	57
Oocatochus rufodorsatus	Squamata	LC	LC	48
Bufo stejnegeri	Anura	LC	LC	46
Elaphe dione	Squamata	LC	LC	43
Rana huanrenensis	Anura	LC	LC	40
Gloydius ussuriensis	Squamata	LC	n/a	28
Lycodon rufozonatus	Squamata	LC	LC	25
Dryophytes japonicus	Anura	LC	LC	20
Glandirana emeljanovi	Anura	LC	LC	20
Bufo sachalinensis	Anura	LC	LC	19
Onychodactylus koreanus	Urodela	LC	n/a	19
Gloydius brevicaudus	Squamata	LC	n/a	13
Bombina orientalis	Anura	LC	LC	11
Hebius vibakari	Squamata	LC	n/a	11
Elaphe schrenckii	Squamata	VU	n/a	10
Hynobius leechii	Urodela	LC	LC	9
Takydromus wolteri	Squamata	LC	n/a	9
Kaloula borealis	Anura	VU	LC	6
Lithobates catesbeianus	Anura	n/a	LC	5
Gloydius intermedius	Squamata	LC	LC	5
Sibynophis chinensis	Squamata	EN	LC	3
Rana coreana	Anura	LC	LC	3
Dryophytes suweonensis	Anura	EN	EN	2
Pelophylax chosenicus	Anura	VU	VU	2
Orientocoluber spinalis	Squamata	NT	n/a	1
Takydromus amurensis	Squamata	LC	n/a	1
Scincella vandenburghi	Squamata	LC	LC	1
Mauremys reevesii	Testudines	VU	EN	1
Pelodiscus maackii	Testudines	VU	n/a	1
Chelonia mydas	Testudines	DD	EN	1

The species list is ordered by the recorded number of roadkills. Note that several duplicate observations and an observation of a sea turtle (*Chelonia mydas*) were subsequently excluded to yield a total of 634 observations used in all downstream analyses.

of 11 species of snakes (all species except *S. chinensis*), one lizard species (*T. amurensis*), one native freshwater turtle species (*P. maackii*), and one invasive freshwater turtle species (*Trachemys scripta elegans*). For amphibians, the literature data had recorded roadkills in 10 out of 14 species of anurans (all species except *D. suweonensis*, *D. flaviventris*, *K. borealis*, and *P. chosenicus*), and two out of 10 species of salamanders (*O. koreanus* and *H. leechii*). In total, the literature data detected 59.5% of the terrestrial herpetofaunal diversity of R Korea (25 out of 42 species). The only two nationally threatened species detected in the literature data were the Russian Ratsnake (*E. schrenckii*; VU; $n = 5$) and the Amur Softshell Turtle (*P. maackii*; VU; $n = 1$). The literature data also detected roadkills of the Slender Racer (*O. spinalis*; NT; NIBR, 2019), with higher frequency ($n = 16$) than the iNaturalist data ($n = 1$). There were no roadkill records of globally threatened species reported in the literature.

Comparisons between citizen science and literature data

Considering the taxonomic representation in both citizen science and literature data, anuran amphibians were detected at the highest frequency (iNaturalist: $n = 333$; literature: $n = 7,828$), followed by snakes (iNaturalist: $n = 260$; literature: $n = 2,750$), salamanders (iNaturalist: $n = 28$; literature: $n = 33$), lizards (iNaturalist: $n = 11$; literature: $n = 5$), and turtles (iNaturalist: $n = 2$; literature: $n = 2$; Figure 3). We converted the raw frequency to percentage to apply the same unit of comparison between the two datasets (iNaturalist and literature). Overall, the two datasets were comparable in terms of the percentage of species detected (Figure 4).

Statistical analyses on the citizen science data

In general, the expected levels of activity associated with key ecological aspects were reflected in the monthly roadkill trends of each reptile and amphibian species (Figure 5, 6). Focusing on the analyses based on the ecological groupings of amphibians, the roadkill trends were significantly different between months for both spring breeding ($n = 173$; $\chi^2 = 231.08$; df = 10; $p < 0.001$) and summer breeding ($n = 123$; $\chi^2 = 116.94$; df = 10; $p < 0.001$) amphibians. For both groups, the highest peak of roadkill records was in September and October. Regarding the Order-level groupings for reptiles, only the squamate grouping had the sufficient sample size at the Order level for statistical analysis, and the number of monthly roadkill observations differed significantly between seasons for this grouping ($n = 271$; $\chi^2 = 266.3$; df = 10; $p < 0.001$), with the peak of roadkill observations in October. The data analyzed at the Class level (Amphibia and Reptilia) also suggested a significant difference in the number of monthly roadkill observations for amphibians ($n = 361$; $\chi^2 = 446.53$; df = 10; $p < 0.001$) and reptiles ($n = 273$; $\chi^2 = 265.27$; df = 10; $p < 0.001$), with peaks of roadkill records in October and September, respectively.

The two peaks of roadkill frequency generally matched with the two peaks of total observations for reptiles. However, for amphibians, the two peaks of total observations were decoupled with a peak of roadkill occurrences in October (Figure 7). The yearly sampling effort of the iNaturalist data, as expressed by the yearly number of observations divided by the yearly number of participants, fluctuated through the years 2005 to 2021. Nevertheless, the overall sampling intensity showed a steadily increasing trend (Figure 8).

Regional sampling intensity

The kernel density maps generated from amphibian and reptile roadkill observation points revealed considerable spatial bias of the iNaturalist dataset (Figures 2C,D). Regarding the amphibian roadkills from the iNaturalist database, most of R Korea had an intermediate sampling intensity. The regions with low sampling intensity were generally located in the southwestern provinces and offshore islands. Meanwhile, the area of the highest sampling intensity was located in the east-central region of R Korea. Regarding the reptile roadkills, the regions with low sampling intensity based on the iNaturalist data were in the southeastern provinces. The regions with high sampling intensity were located along the central R Korea, with the west-central region showing the highest sampling intensity. Other regions fell within the range of intermediate sampling intensity. The spatial sampling bias of herpetofauna roadkills based on the literature dataset was distinctly different from that based on the iNaturalist dataset. Based on the literature data, the survey intensity (measured by provincial "counts") were the highest in the eastern and central provinces (6 ∼ 7 counts), followed by the southern provinces (∼ 4 counts), and the western provinces (∼ 2 counts). Some provinces were either not surveyed, or there were no published literature that surveyed those provinces.

Roadkills across elevation

In the iNaturalist data, the frequency of roadkill records was significantly different across elevational ranges for both amphibians ($n = 292$; $\chi^2 = 91.105$; df = 9; $p < 0.001$) and reptiles ($n = 272$; $\chi^2 = 550.11$; df = 8; $p < 0.001$). The highest number of amphibian roadkills was recorded between 300 and 399 m asl. ($n = 69$), followed by 0–99 m asl ($n = 54$), 200–299 m asl ($n = 45$), 400–499 m asl ($n = 44$), 500–599 m asl ($n = 43$), 100–199 m asl ($n = 39$), 700–799 m asl ($n = 24$), 600–699 m asl ($n = 23$), 900–1,241 m asl ($n = 12$), and 800–899 m asl ($n = 8$). Meanwhile, the highest number of reptile roadkills was recorded between 0 and 99 m asl ($n = 147$), followed by 100–199 m asl (n

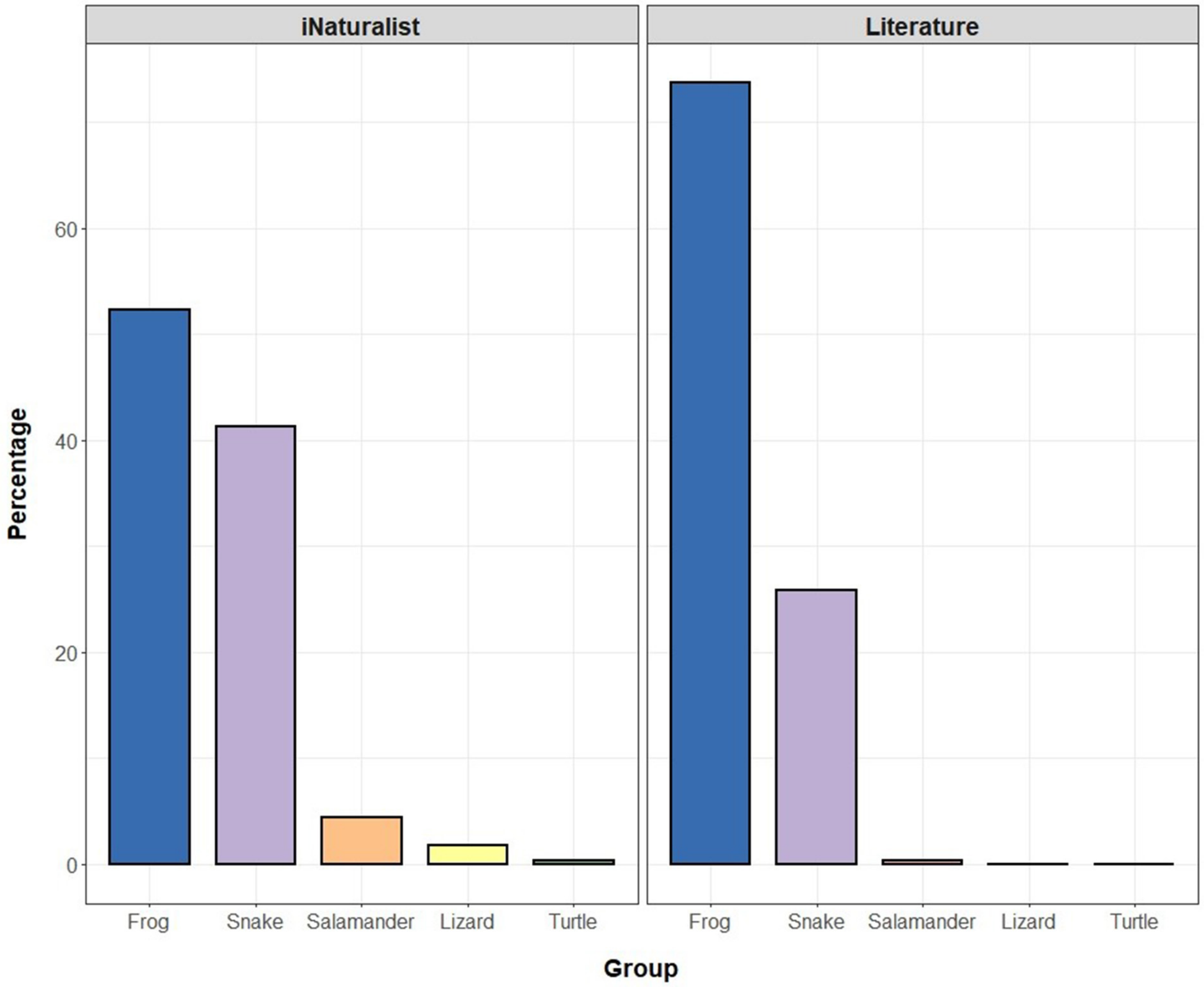

FIGURE 3
Taxonomic sampling of roadkill records according to the citizen science (iNaturalist) and literature data. The overall taxonomic representation, expressed as percentages was similar between the datasets, despite the much larger total sample size of the literature data. The anuran amphibians showed the highest roadkill frequency (iNaturalist: $n = 333$; literature: $n = 7{,}828$), followed by snakes (iNaturalist: $n = 260$; literature: $n = 2{,}750$), salamanders (iNaturalist: $n = 28$; literature: $n = 33$), lizards (iNaturalist: $n = 11$; literature: $n = 5$), and turtles (iNaturalist: $n = 2$; literature: $n = 2$).

= 39), 300–399 m asl ($n = 26$), 200–299 m asl ($n = 25$), 400–499 m asl ($n = 18$), 600–699 m asl ($n = 7$), 500–599 m asl ($n = 5$), 700–799 m asl ($n = 4$), and 800–810 m asl ($n = 1$). Therefore, according to the iNaturalist data, the number of herpetofauna roadkill observations generally showed a decreasing trend with increasing elevation (Figure 9).

Regarding the literature data, the distribution of roadkill records was also significantly different across elevational ranges for both amphibians ($n = 1{,}617$; $\chi^2 = 2{,}592$; df = 7; $p < 0.001$) and reptiles ($n = 802$; $\chi^2 = 586.85$; df = 10; $p < 0.001$; Figure 9). The highest number of amphibian roadkills was recorded from 700 to 799 m asl ($n = 846$), followed by 500–599 m asl ($n = 241$), 300–399 m asl ($n = 222$), 200–299 m asl ($n = 134$), 100–199 m asl ($n = 81$), 600–699 m asl ($n = 40$), 400–499 m asl ($n = 35$), and 0–99 m asl ($n = 18$). For reptiles, the highest number of roadkills was recorded from 100 to 199 m asl ($n = 173$), followed by 200 –299 m asl ($n = 166$), 600–699 m asl ($n = 134$), 400–499 m asl ($n = 117$), 700–799 m asl ($n = 98$), 800–899 m asl ($n = 30$), 0–99 m asl ($n = 25$), 300–399 m asl ($n = 24$), 900 –999 m asl ($n = 15$), 500–599 m asl ($n = 10$), and 1,000 m + asl ($n = 10$). Therefore, according to the literature data, the amphibian roadkill frequency generally showed an increasing trend with increasing elevation, while the reptile roadkill frequency showed a decreasing trend with increasing elevation (Figure 9).

The sample size of NIE data was insufficient to test for differences in the elevational distribution of amphibian roadkills ($n = 10$), although most roadkills were recorded in elevational ranges of 0–99 m asl ($n = 4$) and 500–599 m asl ($n = 3$). On

FIGURE 4
Comparison of the taxonomic composition of roadkill observations between the iNaturalist and literature-based data. Due to the different sample sizes between data types, we converted the raw count of frequency to percentages to apply the same scale of comparison. The most heavily affected species were generally congruent between datasets, with several differences associated with taxonomic diversity detected in each dataset as well as the sampled percentage of each species. Note that the iNaturalist data detected roadkills in more species ($n = 32$; 76.2% of total herpetofaunal diversity of R Korea) compared to the literature-based data ($n = 25$; 59.5% of total herpetofaunal diversity of R Korea). The silhouette figures were obtained from PhyloPic (http://phylopic.org/) under the Public Domain Dedication 1.0 license or the Public Domain Mark 1.0 license (for the turtle silhouette). Frog silhouette (*Leptodactylus melanonotus*) by Jose Carlos Arenas-Monroy; Salamander silhouette (*Onychodactylus fischeri*) by zoosnow; Snake silhouette (*Vipera berus*) by Beth Reinke; Lizard silhouette (*Eumeces fasciatus*) by David Orr; Turtle silhouette (*Chrysemys picta*) by an uncredited author.

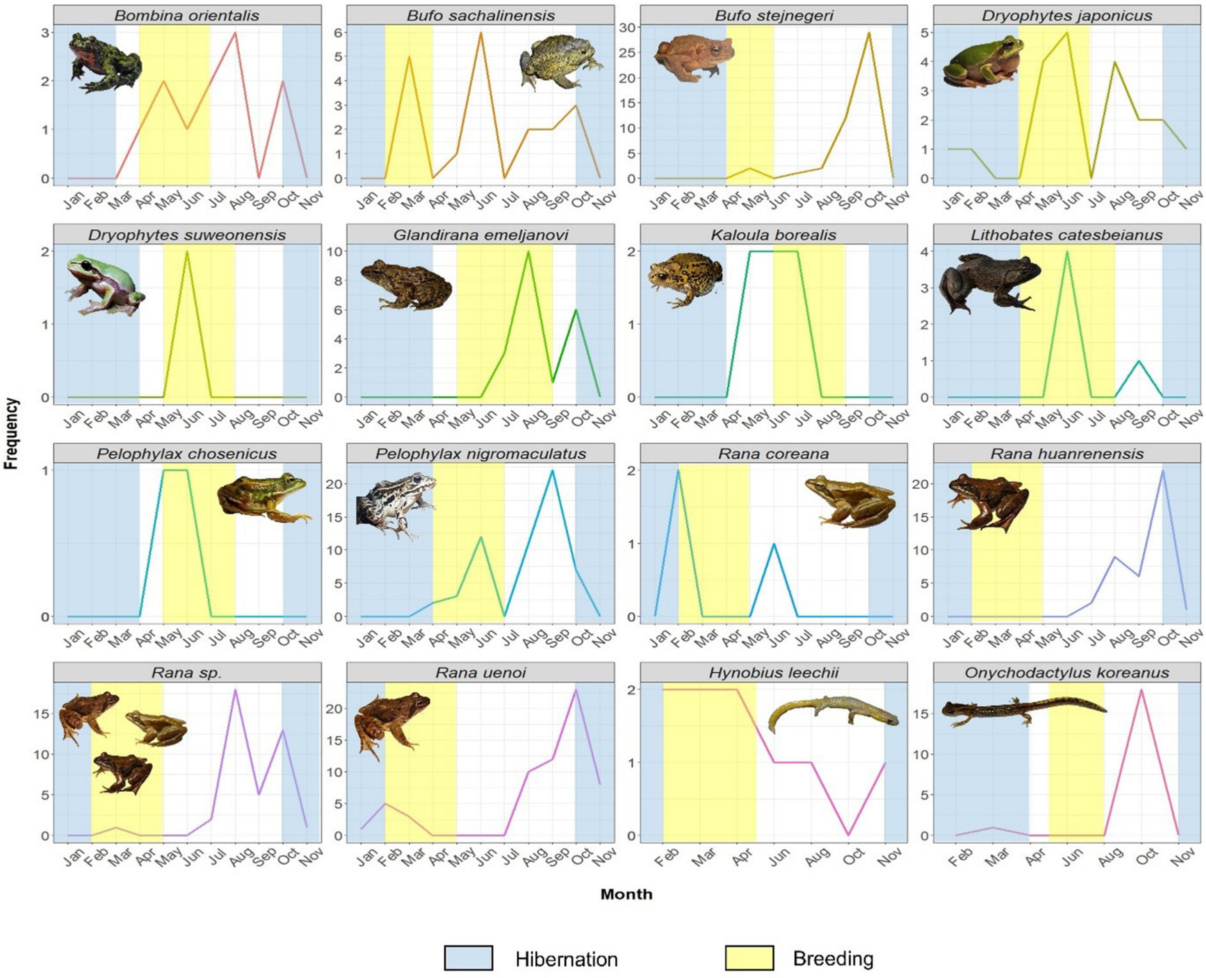
Bombina orientalis
Bufo sachalinensis
Bufo stejnegeri
Dryophytes japonicus
Dryophytes suweonensis
Glandirana emeljanovi
Kaloula borealis
Lithobates catesbeianus
Pelophylax chosenicus
Pelophylax nigromaculatus
Rana coreana
Rana huanrenensis
Rana sp.
Rana uenoi
Hynobius leechii
Onychodactylus koreanus

FIGURE 5
The monthly roadkill trends for Korean amphibians ($n = 361$), based on iNaturalist observations. The roadkill records were collected from 1 January 2005 to 13 August 2021 through the iNaturalist project "Herpetofauna Roadkill in the Republic of Korea". Ambiguous observations with identifications above the genus level were excluded. The information on breeding season and hibernation period for each species is based on Lee and Park (2016), and summarized in Supplementary Table 2. Photographs of amphibians by AB.

the other hand, the distribution of NIE reptile roadkill records was significantly different across elevational ranges ($n = 134$; $\chi^2 = 78.776$; df = 7; $p < 0.001$; Figure 9). The highest number of reptile roadkills was recorded in 0–99 m asl ($n = 36$), followed by 100–199 m asl ($n = 34$), 300–399 m asl ($n = 26$), 200–299 m asl ($n = 18$), 400–499 m asl ($n = 6$), 500–599 m asl ($n = 6$), 600–699 m asl ($n = 6$), and 800–899 m asl ($n = 2$).

Roadkills across habitat types

The distribution of iNaturalist roadkill records was significantly different across habitat types, for both amphibians ($n = 325$; $\chi^2 = 540.79$; df = 5; $p < 0.001$; Figure 10) and reptiles ($n = 260$; $\chi^2 = 287.29$; df = 6; $p < 0.001$; Figure 10). The highest number of amphibian roadkills was recorded in forest environments ($n = 198$), followed by agriculture ($n = 84$), grassland ($n = 20$), residential area ($n = 14$), barren ground ($n = 5$), and water bodies ($n = 4$). Meanwhile, the highest number of reptile roadkills was recorded in agricultural landscapes ($n = 106$), followed by forest ($n = 90$), wetland ($n = 21$), residential area ($n = 15$), barren ground ($n = 14$), grassland ($n = 11$), and water bodies ($n = 3$).

The distribution of literature-based roadkill records was also significantly different across habitat types, for both amphibians ($n = 1{,}750$; $\chi^2 = 1311.7$; df = 4; $p < 0.001$) and reptiles ($n = 966$; $\chi^2 = 1941.8$; df = 7; $p < 0.001$; Figure 10). For amphibians, the highest number of roadkills was recorded from the intersection of forest environments (forest-forest; $n = 724$), followed by the intersection of agricultural landscapes (agriculture-agriculture; $n = 633$), intersection of forests and water bodies (forest-water; $n = 383$), intersection of agricultural

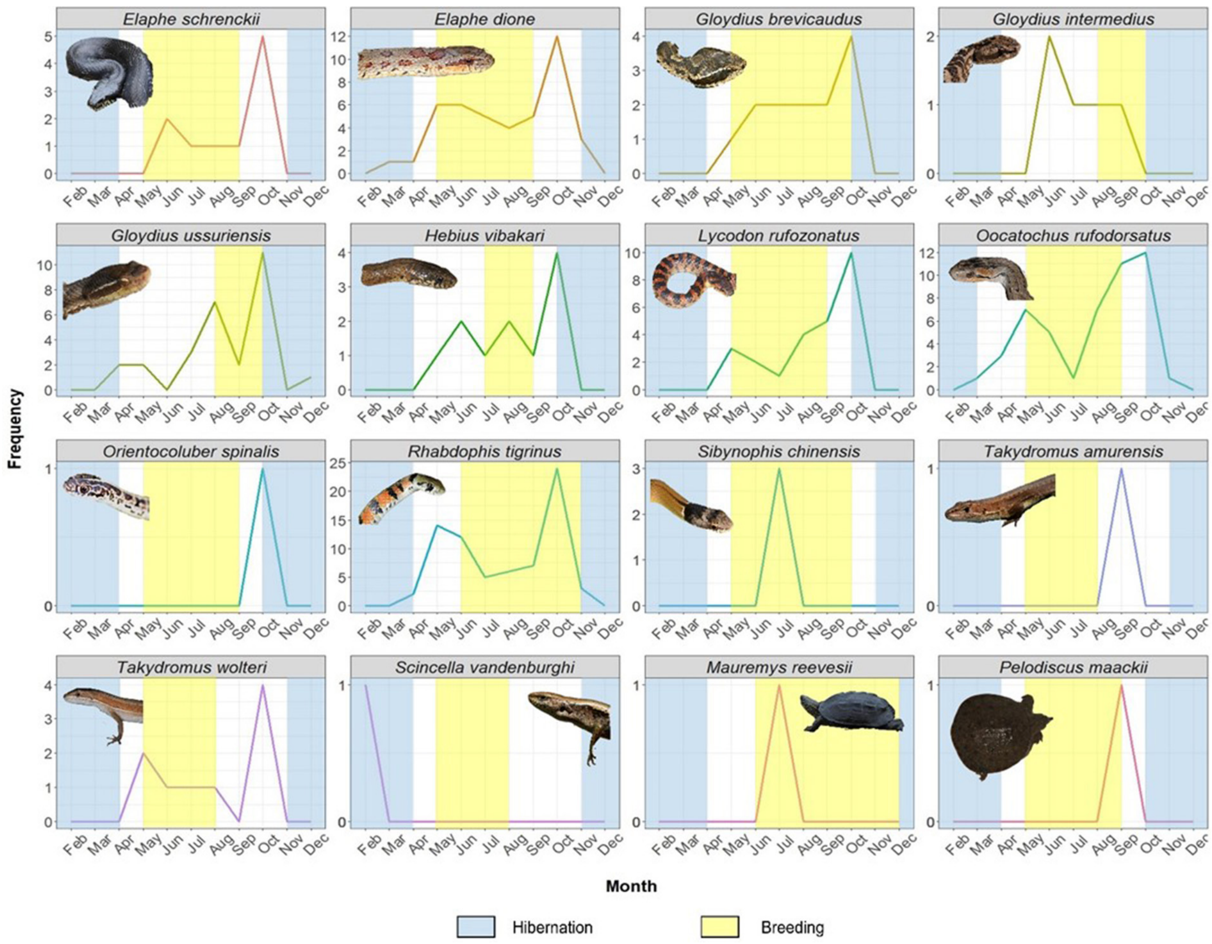

FIGURE 6
The monthly roadkill trends for Korean reptiles based on iNaturalist observations ($n = 273$). The roadkill records were collected from 1 January 2005 to 13 August 2021 through the iNaturalist project "Herpetofauna Roadkill in the Republic of Korea". We excluded observations with ambiguous identifications above the species level. The information on breeding and hibernation periods are primarily derived from Lee et al. (2012). The breeding season of *Sibynophis chinensis* was inferred from Koo et al. (2018) and Banjade et al. (2020), whereas information on the breeding season of *Gloydius intermedius* was further supplemented from Do et al. (2017). Photographs of reptiles courtesy of Hyun-Tae Kim, modified and used under permission.

landscapes and residential areas (agriculture-residential; $n = 6$), and the intersection of forests and agricultural landscapes (forest-agriculture; $n = 4$). Regarding reptiles, the highest number of roadkills was recorded from the intersection of forests and water bodies (forest-water; $n = 522$), followed by the intersection of forest environments (forest-forest; $n = 252$), the intersection of forests and agricultural landscapes (forest-agriculture; $n = 108$), the intersection of agricultural landscapes (agriculture-agriculture; $n = 50$), the intersection of agricultural landscapes and water bodies (agriculture-water; $n = 31$), the intersection of grasslands and forests (grassland-forest; $n = 1$), the intersection of residential areas (residential-residential; $n = 1$), and the intersection of water bodies and residential areas (water-residential; $n = 1$; Figure 10).

The sample size of NIE data was insufficient to test for the differences in habitat distribution of amphibian roadkills ($n = 10$), although most roadkills were recorded in forests ($n = 4$) and agricultural landscapes ($n = 3$). Meanwhile, the distribution of NIE reptile roadkill records was significantly different in habitat types ($n = 61$; $\chi^2 = 31.841$; df $= 4$; $p < 0.001$). The highest number of reptile roadkills was recorded in forests ($n = 23$), followed by agricultural landscapes ($n = 22$), residential areas ($n = 13$), grasslands ($n = 3$), and near water bodies ($n = 2$; Figure 10).

We measured and compared the distance between all roadkill points and the seven land cover types. The Bayesian inference for the Linear Regression Model showed that the distances between roadkill observations and the seven land cover types were different between reptiles and amphibians (Bayes Factor = 0.00, R = 0.17; $R^2 = 0.03$, SE = 237.27). The average distance between roadkill observations and wetlands was nearly twice higher in amphibians than reptiles (2,023 m

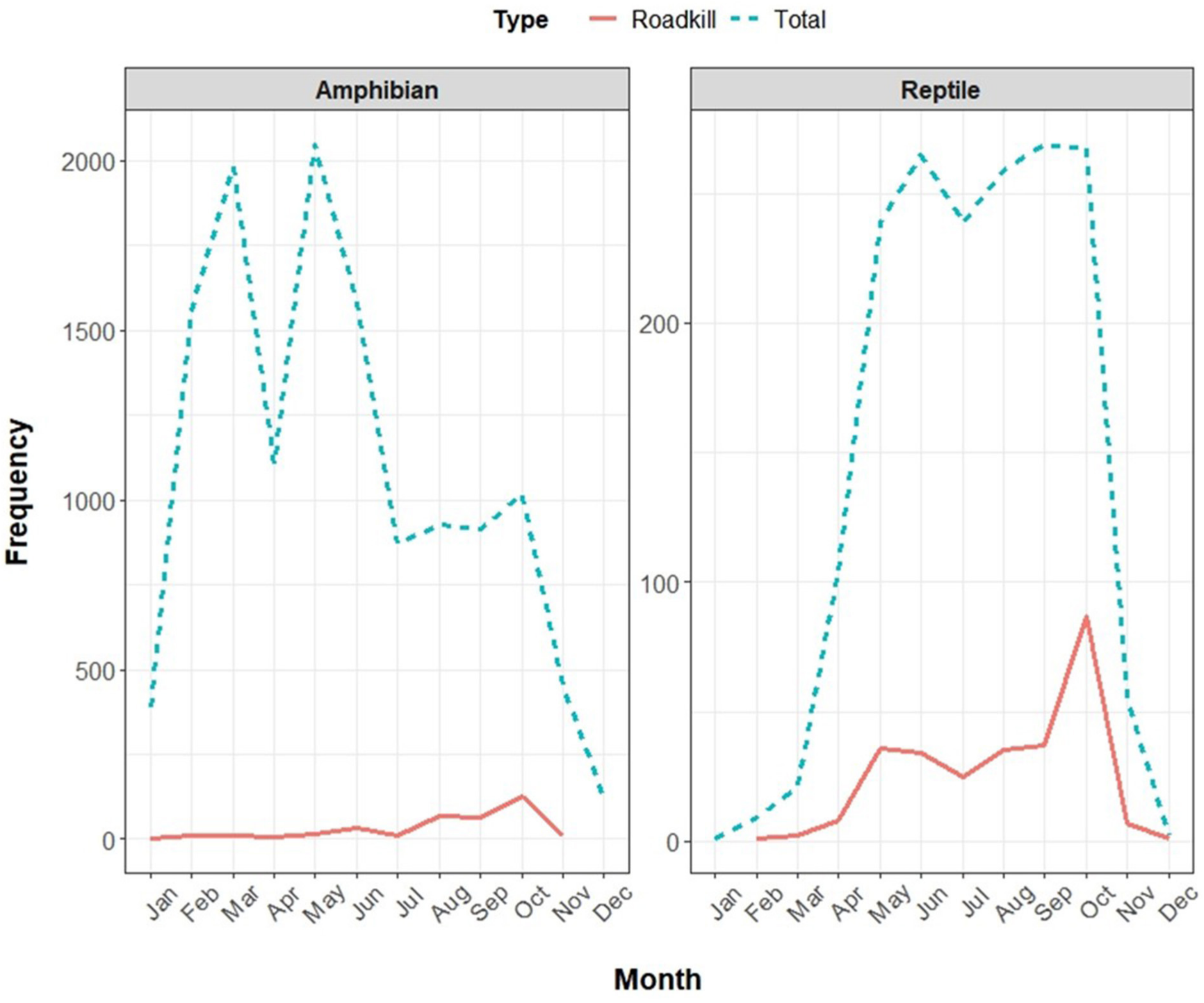

FIGURE 7
The total monthly roadkill records for the Korean herpetofauna (total $n = 634$; amphibian $n = 361$; reptile $n = 273$) plotted against the total monthly observations (total $n = 14{,}699$; amphibian $n = 12{,}969$; reptile $n = 1{,}730$), based on the pooled iNaturalist dataset collected from 1 January 2005 to 13 August 2020. Distinctly different monthly trends are notable between the total observations and roadkill observations for both amphibian and reptile datasets, highlighting that the peaks in the number of roadkills do not match the peaks in the number of total observations.

for amphibians and 1,021 m for reptiles). In addition, the three habitat types the closest to the occurrences of roadkills were agricultural lands (48.82 ± 104.06 m; mean ± SD), wetlands (86.02 ± 142.47 m), and forests (216.86 ± 210.41 m) for amphibians, and agricultural lands (29.49 ± 94.78 m), forests (91.23 ± 148.04 m), and grasslands (200.51 ± 231.70 m) for reptiles.

Discussion

Our results demonstrate that the citizen science data can detect roadkills in the majority of the Korean terrestrial herpetofauna with broad geographic coverage and can capture patterns related to the ecology of individual species given sufficient sample size. Therefore, citizen science-based roadkill surveys have significant benefits that can complement traditional survey methods. Here we show that the iNaturalist roadkill dataset is capturing the key points of the behavioral ecology of the Korean herpetofauna in terms of monthly observation trends, habitat use, and especially for species with large sample sizes (Figure 5, 6 and Supplementary Figures 1, 2). In general, the peak(s) of roadkill for each species of reptiles and amphibians coincided with the periods immediately before the breeding season, during the breeding seasons, and periods immediately before the hibernation period. Increased movements can be expected in many reptile and amphibian species during these periods, as they often migrate long

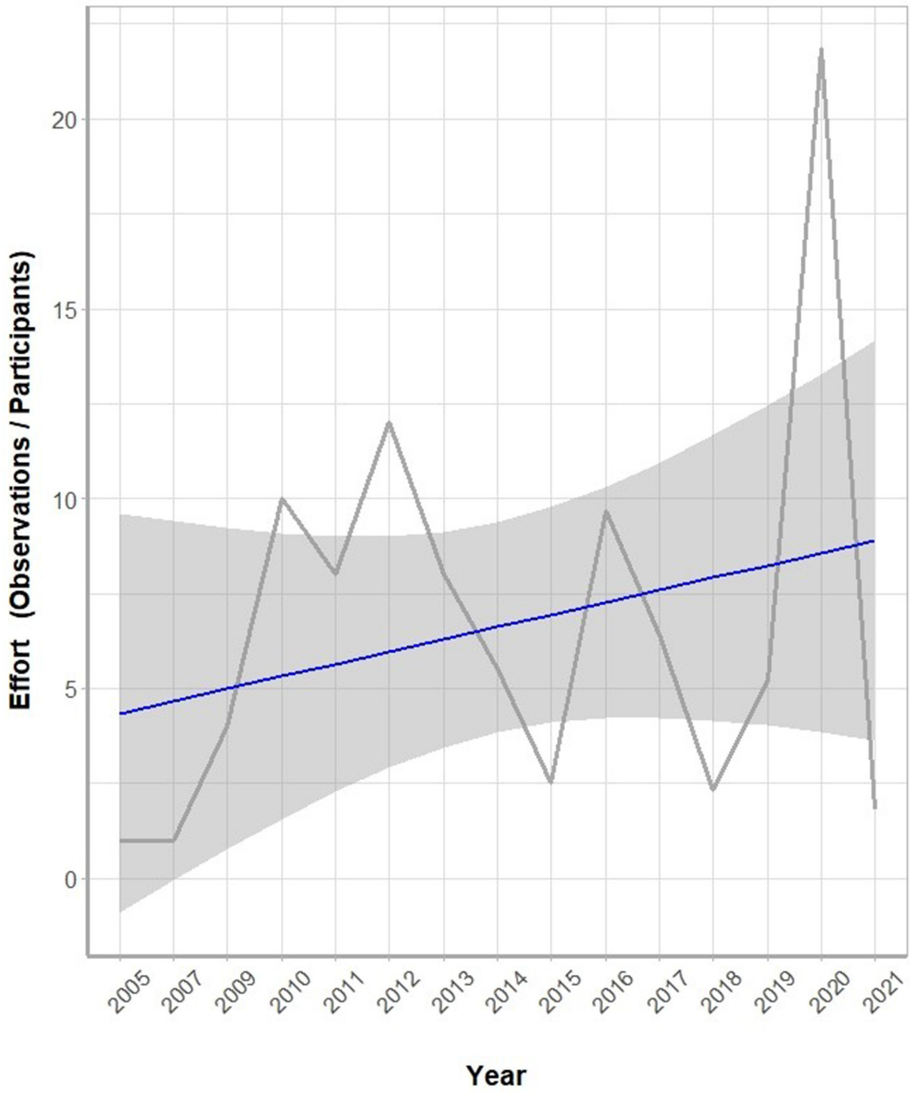

FIGURE 8
The yearly sampling efforts of the iNaturalist roadkill observations through the years 2005 to 2021 (from 1 January 2005 to 13 August 2020). Here, the yearly sampling effort is expressed as the number of observations per year divided by the number of participants per year. Overall, the sampling efforts show an increasing trend through the years. The trend line (blue) is shown with 95% confidence interval.

distances in search of breeding habitats or suitable hibernacula. For example, the frequency of total monthly observations captured two life history aspects for the three *Rana* species (plus a non-identifiable group of "*Rana* sp." Supplementary Figure 1). The peaks in monthly roadkill observations between February and March also correspond to the breeding season (Figure 5), as this is the period when adults emerge from hibernation and migrate over the landscape to reach their breeding habitats (Song et al., 2009; Park et al., 2017). In addition, the lack of roadkill observations after the breeding seasons followed by a second peak of roadkill observations in October (mostly composed of *R. uenoi* and *R. huanrenensis*), correspond to the adults migrating to their hibernacula across roads. The iNaturalist data also captured species-specific roadkill trends in amphibian species with highly specialized ecologies. For instance, the frequency of roadkills in *B. stejnegeri*, as well as total monthly observations, was the highest immediately before hibernation, rather than during the breeding season as in most other amphibian species (Figure 5 and Supplementary Figure 1). This is likely because *B. stejnegeri* hibernates underwater as amplectant pairs and breed as they emerge from hibernation without long-distance migration to breeding habitats. Therefore, the roadkill patterns of *B. stejnegeri* are generally consistent with the patterns for other amphibian species while also reflecting

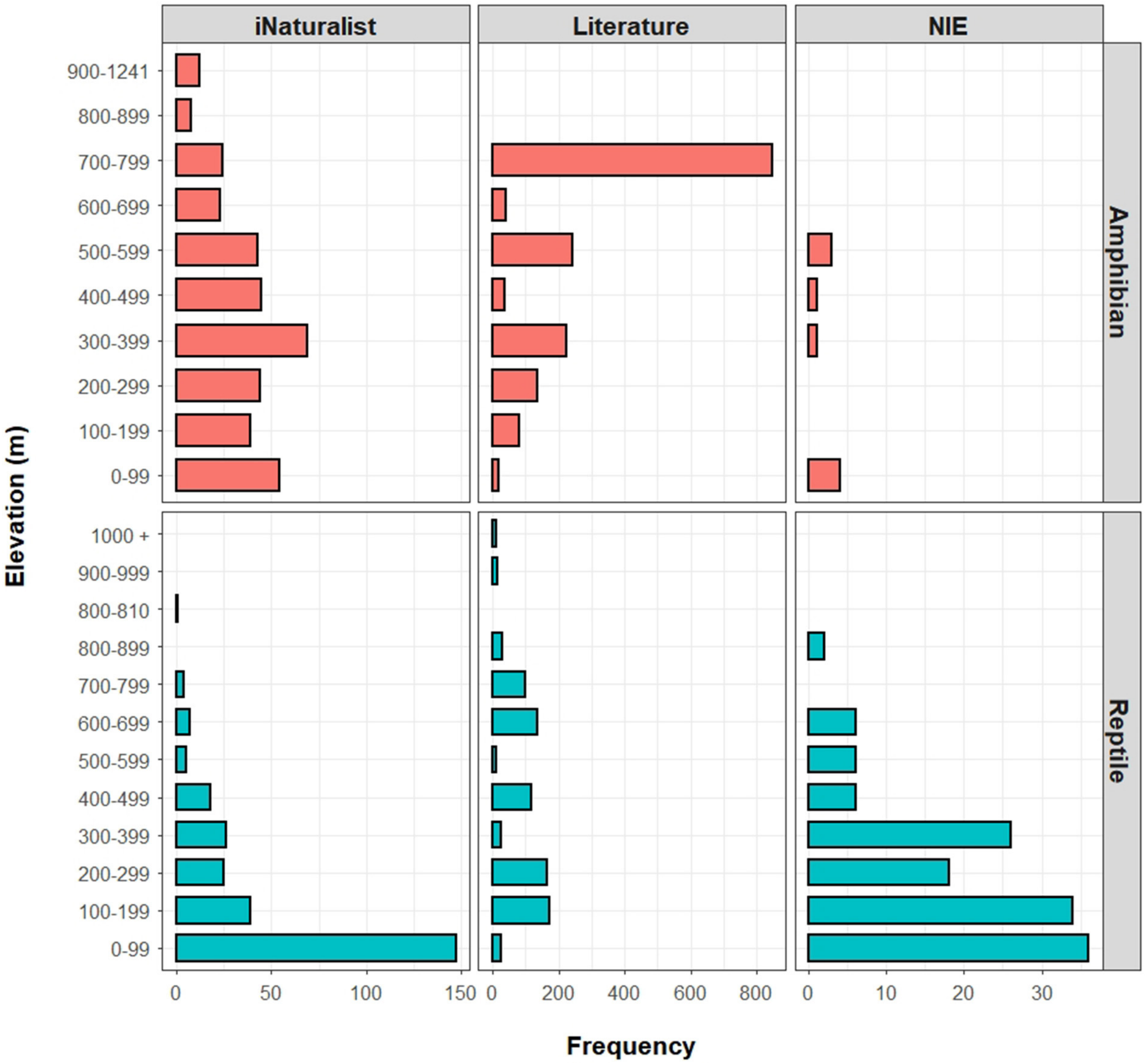

FIGURE 9
Elevational distributions of roadkill records compared among the iNaturalist, literature, and governmental (National Institute of Ecology; NIE) data. Regarding the iNaturalist data, the amphibian roadkill frequency was the highest around low to mid elevations, whereas the reptile roadkill frequency was the highest in low elevations, with clear decrease toward higher elevations. The literature-based data shows a different trend, with amphibian roadkill frequency roughly increasing toward higher elevations, and with reptile roadkill frequency showing fluctuating patterns. The sample size of NIE data is insufficient to detect elevational patterns for amphibians, but there is an overall decreasing trend with increasing elevation for reptiles.

aspects of the ecology specific to the species. However, outside the breeding period, *B. stejnegeri* migrates back to summer habitats in the mountains. Therefore, the lack of a second peak of recorded roadkills in the summer (as expected from the ecology of species) likely reflects the low sampling efforts in this period (refer to the paragraph below).

Both species-specific behavioral ecology and the sampling mode can explain why some species were not detected in the iNaturalist dataset. For some species, there are clear biases stemming from the lack of sampling. For instance, *H. leechii* was the only *Hynobius* species detected by both the iNaturalist and literature datasets, even though there are seven species of *Hynobius* described in R Korea (Borzée and Min, 2021). *H. leechii* is the most common and widely distributed species of the genus in R Korea and it migrates across roads in large numbers during the breeding season (A Borzée, pers. obs.), which is partly reflected in the peak of roadkill records in the iNaturalist data. However, as only a few roadkills of this species were detected, the true magnitude of roadkill affecting this species is likely to be greatly underrepresented in the iNaturalist data. On the

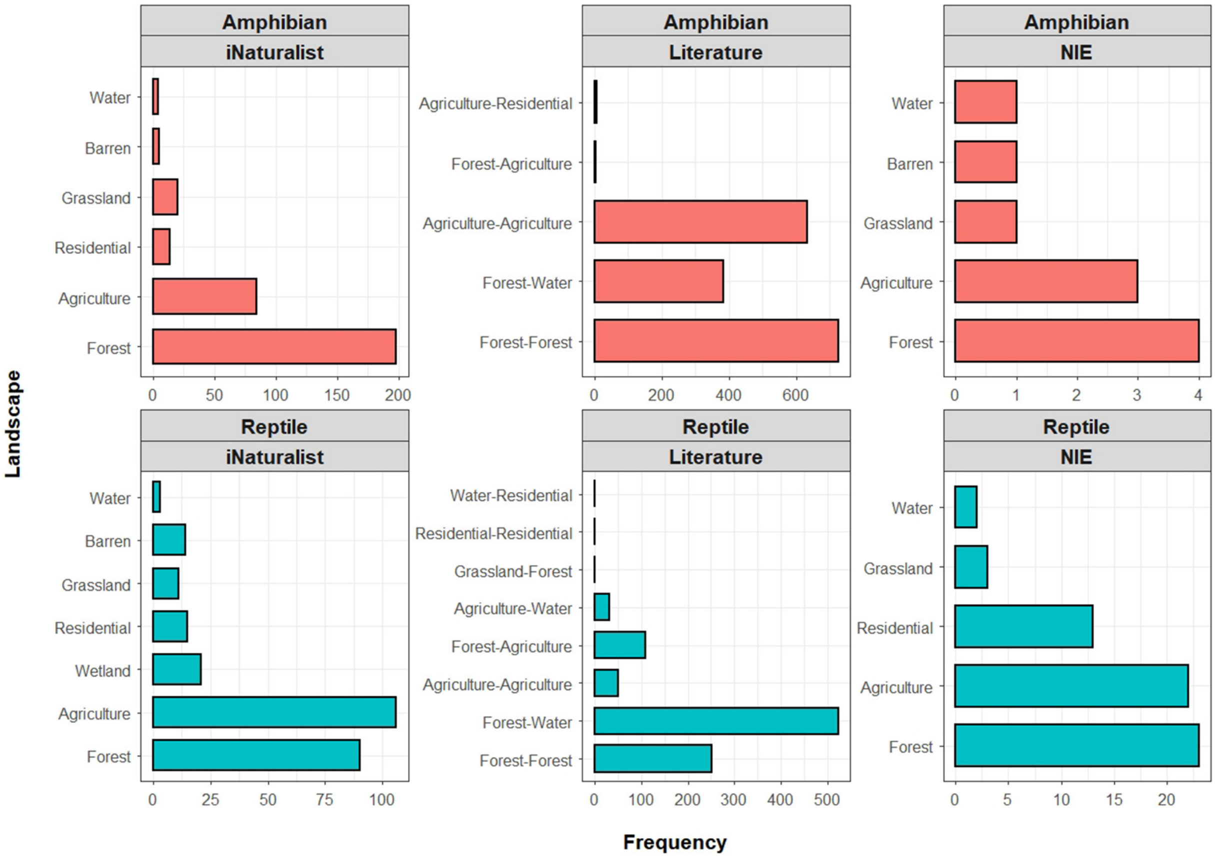

FIGURE 10
Distribution of roadkill records across different habitat types compared among the iNaturalist, literature, and governmental (National Institute of Ecology; NIE) data. While the specific classification of habitat types differ between datasets, all three datasets generally converge on a congruent pattern by detecting the highest frequency of herpetofauna roadkills in either forest or agricultural landscapes.

other hand, all seven species of *Hynobius* in R Korea form a clade of lentic breeders with similar ecologies (Borzée and Min, 2021). However, the other species of the genus less common than *H. leechii* have a relatively small distribution range and are found in more rural areas, which in combination with their secretive lifestyles and habitat characteristics might reduce the potential risk of roadkill and explain the lack of records. This low representation of the species is further supported by the spatial distribution of sampling intensity across R Korea (southern R Korea; Figure 2C). The same pattern is also found in other rare species with restricted ranges, such as the Critically Endangered treefrog *D. flaviventris* (Pers. Obs.; Borzée et al., 2020).

Habitat characteristics of roadkills were different for amphibians and reptiles, although agricultural lands and forests were among the top three habitat types where roadkills were recorded for both classes. This similarity at the macro scale despite the difference is likely related to the broadly similar habitat use of many reptile and amphibian species. Habitat characteristics could also explain the lack of roadkill detection for the three lizard species (*Eremias argus*, *Gekko japonicus*, and *Scincella huanrenensis*). For instance, *G. japonicus* has a restricted distribution in the southern coastal regions (Kim et al., 2017a) and is usually observed on walls, fences, and roofs in city parks and residential areas (Lee et al., 2012; Kim et al., 2018a). Similarly, *E. argus* have small home ranges (Kim et al., 2012) and they are restricted to coastal sand dunes, inland grasslands along lakes, and mountain slopes with low vegetation cover (Lee et al., 2012). Finally, the distribution of the third species, *S. huanrenensis,* is poorly known although potentially distributed across a broader range than currently known (Shin et al., 2021), and the species is usually found in the forest interiors (Lee et al., 2012). The behavioral characteristics and habitat use of the three lizard species is likely the cause for the reduced risk of roadkill.

Some of the discrepancies in the spatial distributions of herpetofauna roadkills between the iNaturalist and literature datasets originate from apparent sampling biases. For example, the elevational distributions of amphibian roadkills based on the iNaturalist and literature data show a roughly inversed pattern (Figure 9), noting that the elevational distribution from the iNaturalist dataset better represents the actual elevational distribution of the species (Andersen et al., 2022). Although fully disentangling the conflicting patterns between datasets can be difficult due to the characteristics of different data types, differences in sample sizes, and associated sampling artifacts, the reason for the conflicting elevational distribution of amphibian roadkills can be partly attributed to an apparent sampling bias in the literature dataset, but also partly explained by the general scarcity of literature on the herpetofauna roadkill in R Korea. Therein, the high frequency of amphibian roadkills at high elevations mostly originated from a single study (Yang et al., 2006), which recorded an exceptionally large number of roadkills ($n = 568$) for a single species (*B. sachalinensis*) at 700 m + asl, an elevation at which they are not common (Andersen et al., 2022). In addition, due to the large sample size collected through the citizen science project, and the demonstrated match between large citizen science datasets collected on roads and real species distribution for toads in the United Kingdom (Petrovan et al., 2020), we expect the iNaturalist project to be representative of the distribution of the species when the sample size is large enough, and we expect the literature data to be skewed toward the high-value sites selected for the studies.

Importantly, there are also similarities between the iNaturalist and literature datasets. For example, the five snake species with the highest roadkill frequency are generally congruent between the datasets, with *Rhabdophis tigrinus* scoring the highest in both datasets. The same general pattern can be found among anuran species, with *Rana uenoi* having the highest frequency in both data types. However, there were also some notable differences. First, the five snake and anuran species with the highest roadkill frequencies, although generally similar, were not identical between the datasets (Figure 4). For example, *Pelophylax nigromaculatus* has the second highest roadkill frequency recorded for anurans after *R. uenoi* and *Oocatochus rufodorsatus* has the second highest roadkill frequency recorded for snakes after *R. tigrinus* in the iNaturalist dataset. However, both *P. nigromaculatus* and *O. rufodorsatus* have low recorded roadkill frequencies in the literature. This discrepancy can be traced to an apparent under-sampling of agricultural landscapes in standardized surveys (Figure 10). It is also notable that the iNaturalist data detected roadkills in seven more species than the literature data. Moreover, the iNaturalist dataset recorded more roadkills of threatened species than the literature data. For example, *S. chinensis*, *K. borealis*, and *P. chosenicus* were only detected by the iNaturalist data, and *E. schrenckii* was detected more frequently by the iNaturalist data ($n = 10$) than the literature data ($n = 5$).

It is important to note that the frequency of roadkills is linked with detection rate, which is highly dependent on weather conditions (Kosmala et al., 2016), as bad weather impacts the activity of both citizen science and standardized surveys, and good weather impacts the preservation of roadkill before decomposition or scavenging, resulting in variations in body size, color, state of preservation, and potential for identification (Chyn et al., 2019). In addition, while roadkills of juveniles are less likely to be preserved long enough to be detected compared to adults, the roadkills of the *E. schrenckii* were composed mostly of juveniles hatched that same year. The high ratio of juveniles is likely linked to the seasonal dispersal by the species, highlighting that a large enough sample size can compensate the weaknesses of citizen science data. Here, we analyzed terrestrial roadkills, including that of species with aquatic life-stages. However, it is more difficult for citizen science to detect deaths related to human activities in the aquatic habitat, and thus creating a bias in our data. Nevertheless, citizen science can detect deaths of aquatic herpetofauna caused by human activities as the iNaturalist dataset also captured an observation of a dead Green sea turtle (*Chelonia mydas*), likely resulting from a collision with a boat (Denkinger et al., 2013).

Even with its unstandardized nature, spatial bias, and observer bias inherent to the iNaturalist data, or any citizen science dataset, this type of data has considerable advantages and values when investigating the broad-scale trends of roadkill. For example, one distinct advantage of using public platforms such as iNaturalist in roadkill surveys is the ability to make multiple validations on species-level identifications, and the ability to re-evaluate identifications based on photographic data accompanying each uploaded observation. This can increase the confidence of species-level identification, and thereby improve the overall quality of data. On the other hand, such validations are rarely possible for standardized surveys, as only the results of the surveys are reported without reference to photographic data. For instance, any reader is able to confirm or reject the identification of the observations included in this research (https://www.inaturalist.org/projects/herpetofauna-roadkill-in-the-republic-of-korea), which is not possible with other published research. In addition, another significant advantage of citizen science-based roadkill surveys is the ability to rapidly accumulate roadkill data from across broad geographic regions and habitats with minimal resource inputs.

We also highlight the unique and different advantages of citizen science-based roadkill surveys and typical standardized roadkill surveys that can be mutually beneficial. For example, some detailed aspects of roadkill (e.g., the age demography of roadkill) might be difficult to study with citizen science data if these data are not standardized to some degree. This issue of data collection can be overcome by citizen science initiatives by adopting the standardized or semi-standardized survey protocols from typical, standardized roadkill surveys (similar to Gardner et al., 2019). The standardized surveys conducted by professional researchers can also benefit from including the unstandardized citizen science data. The standardized roadkill surveys previously conducted and reported from R Korea were usually conducted at fixed temporal intervals in set routes (Yang et al., 2006; Song et al., 2009; Min and Han, 2010). While these surveys and related studies have provided important insights into the broad patterns of herpetofauna roadkills in R Korea, they were generally limited to certain stretches of roads penetrating key habitats (e.g., National Parks; Song et al., 2009; Cho, 2011; Koo et al., 2018). Therefore, citizen science data can help researchers to make informed decisions on survey locations and timing. For example, the spatial density, habitat, and elevational representations of roadkill sampling in citizen science platforms can be used by researchers to target specific habitat types and/or under-sampled regions to broaden the survey extent. Furthermore, researchers can utilize the citizen science data to specifically target otherwise opportunistic elements of roadkill detections, such as mass migration of amphibians associated with movements to and from breeding sites and hibernacula which are often affected by short-term or even hourly environmental conditions (Shin et al., 2020). Therefore, the advantages of citizen science and standardized survey data can complement each other to better characterize the true magnitude of roadkill affecting the herpetofauna of R Korea.

In conclusion, our study demonstrated citizen science as a viable method to survey roadkill trends of herpetofauna, adding to a growing number of studies highlighting the benefits of citizen science in biodiversity and conservation research. However, the estimates of roadkill trends derived from the citizen science, as well as targeted road surveys, still underestimate the true magnitude, and subsequent impacts, of roadkill to the Korean herpetofauna. Therefore, we encourage the users of citizen science platforms to upload more roadkill observations and encourage researchers to utilize such data to get a fuller picture of the impacts of roadkill affecting the Korean herpetofauna.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was reviewed and approved by Ethic Committee of Nanjing Forestry University.

Author contributions

YS, KK, JG, and AB: conceptualization and visualization. YS and KK: formal analysis. AB: funding acquisition. YS, KK, and JG: writing the original draft. YS, KK, JG, DW, ES, and AB: writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Foreign Youth Talent Program (QN2021014013L) from the Ministry of Science and Technology of the People's Republic of China to AB.

Acknowledgments

We are grateful for the efforts of the citizen scientists who entered herpetofauna roadkill observations to iNaturalist, and especially @pintail for their numerous observations. We also thank the contributors of the PhyloPic silhouettes database. Finally, we thank the handling editor Dr. Silviu O. Petrovan and two reviewers for providing constructive comments that significantly improved this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.944318/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fevo.2022.958655

Improving trajectories of amphibians in wildlife passages

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SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 31 May 2022
ACCEPTED 26 September 2022
PUBLISHED 20 October 2022

CITATION
Testud G, Canonne C, Le Petitcorps Q, Picard D, Lengagne T, Labarraque D and Miaud C (2022) Improving trajectories of amphibians in wildlife passages.
Front. Ecol. Evol. 10:958655.
doi: 10.3389/fevo.2022.958655

Linear transport infrastructure can alter the viability of populations and wildlife passages are used to mitigate their impacts. The assessment of their outcomes is often limited to recording the use of the tunnels by a focal species. For amphibians, the effectiveness of tunnels is poorly evaluated with little information about whether certain features encourage individuals that may be reluctant to pass through tunnels. One study showed that acoustic enrichment with anuran calls can increase the crossing of tunnels by newts. This study recorded the behavior of three European amphibian species in three tunnels, tracking them with PIT tags and detection with four RFID antennas installed on the floor of the tunnels. We tested (1) the effectiveness of the antennas in detecting the species, (2) the effect of the length of the tunnels, and (3) the effect of acoustic enrichment. Using a multi-state capture–recapture model, we evaluated the probability of an individual advancing between the tunnel sections. The effectiveness of the antennas varied according to species, higher for Urodela species than for Anuran species. Several types of paths were detected (constant and varying speeds, halt, and back-and-forth movements). The fire salamander and the great crested newt individuals exhibited a similar variety of movements in the tunnels (21 and 40 m length). *Triturus cristatus* made similar movements in the tunnels with and without acoustic enrichment. In water frogs, all the individuals (n = 16) made a complete crossing in the tunnel with enrichment vs. 75% (n = 71) in the tunnel without enrichment. In *T. cristatus*, the probability of going forward at the entrance of the tunnel was 18% higher with enrichment in one tunnel. No significant effect of acoustic enrichment was observed in two others tunnels for this species. In *Pelophylax esculentus*, this probability was 78% higher in the tunnel with enrichment. This multi-antenna RFID system was able to provide valuable information on the behavior of these small animals when traversing the tunnels, as well as to test the effectiveness of tunnel features. The findings indicate that acoustic enrichment to attract animals to specific locations holds promise as a new conservation tool.

KEYWORDS
linear transport infrastructure, amphibian, wildlife passage, acoustic enrichment, multi-state capture–recapture model

Introduction

Linear transportation infrastructure (LTI) such as roads and railways are one of the major anthropogenic alterations to the world's ecosystems (Rytwinski and Fahrig, 2015; Popp and Boyle, 2016). They can have a number of negative impacts on wildlife, including habitat loss, habitat fragmentation, loss of connectivity, and direct and indirect mortality (Forman and Alexander, 1998; Schmidt and Zumbach, 2008; Rytwinski and Fahrig, 2015). While most studies on LTI have focused on roads, railways have theoretically similar effects (Bartoszek and Greenwald, 2009; Dorsey, 2011; Popp and Boyle, 2016), even with lower traffic (Rodriguez et al., 1996). Yet to date there has been a lack of studies on railway ecology (Dorsey, 2011; Popp and Boyle, 2016; Testud and Miaud, 2018), especially on reptiles and amphibians (Kornilev et al., 2006; Budzik and Budzik, 2014; Kaczmarski and Kaczmarek, 2016).

Conservation planners attempt to use knowledge of species biology and the types of impacts identified to propose mitigation measures (Testud and Miaud, 2018). Wildlife passages such as tunnels for small vertebrates (e.g., amphibians) and fences are today commonly implemented in LTI planning and construction (Jarvis et al., 2019; Schmidt et al., 2020). While these are assumed to improve the viability of impacted populations (Clevenger and Ford, 2010; Hamer et al., 2014; Jarvis et al., 2019), the evaluation is complex and costly in terms of budget, time and manpower and is often limited to recording tunnel use (e.g., Puky et al., 2007; Pagnucco et al., 2012; Matos et al., 2017; Testud and Miaud, 2018). The political agenda is often too shortsighted (Lesbarrères and Fahrig, 2012). The behavior of amphibians encountering a wildlife crossing (Matos et al., 2018) and fences (Brehme et al., 2021) has been rarely studied (Conan et al., 2022).

It is known that individuals can be reluctant to cross tunnels (Allaback and Laabs, 2003; Bain et al., 2017; Matos et al., 2017, 2018; Jarvis et al., 2019), and better knowledge on the causes of avoidance would be particularly valuable for designing new tunnels or adapting existing structures (Brehme et al., 2021). Sensory-based conservation harnesses species' natural communication and signaling behavior to mitigate threats to wild populations (Friesen et al., 2017). Wildlife managers have begun employing acoustic attraction to lure animals to specific places by broadcasting vocalizations (Putman and Blumstein, 2019). Playbacks are frequently used to attract birds to unoccupied sites or to maintain animals in a new translocation site (Ward and Schlossberg, 2004; Molles et al., 2008) and thus are recommended for conservation and restoration (Ahlering et al., 2010; Friesen et al., 2017; Znidersic and Watson, 2022). In amphibians, using conspecific chorus calls has been shown to improve colonization (Buxton et al., 2015; James et al., 2015). A study has also shown that acoustic enrichment of tunnels with frog calls increased the speed and complete crossing rates of water frogs (*Pelophylax esculentus*) and the great crested newt (*Triturus cristatus*) (Testud et al., 2020). Individuals with PIT tags tracked by RFID antennas deployed in tunnels can give detailed information on movements inside the tunnel (Testud et al., 2019).

In this study, we investigate the movement behavior of three European amphibian species during tunnel crossings, with individuals tracked by PIT tags and RFID antennas. The complete crossing or non-crossing by a U-turn or other behaviors as well as the crossing speeds were presented in Testud et al. (2020). The experimental design allowed the description of the trajectories (direction changes, variations in speed) of the great crested newt (*T. cristatus)*, fire salamander (*Salamandra salamandra)*, and water frog (*P. esculentus*) in tunnels of varying lengths. We also broadcasted a mixed chorus of the water frog (*P. esculentus)* and the European tree frog (*Hyla arborea)* in tunnels in order to test the effects of this acoustic enrichment on the individual trajectories of the water frog (*P. esculentus*) and the great crested newt (*T. cristatus)*. Multi-state models were used to evaluate the effect of acoustic enrichment on the estimated probability of the amphibians moving forward at successive positions in tunnels.

Materials and methods

Study area and wildlife crossing structure

Three wildlife crossing structures built under a high-speed railway (HSR) line were studied in the Pays-de-la-Loire region in northwestern France. The three tunnels and surrounding landscape are described in Testud et al. (2020). Two tunnels are located close to the small town of Chantenay-Villedieu, at a distance of 800 m from each other (Tunnels 1 and 2). The third is located close to the small town of Beaulieu-sur-Oudon, 70 km away (Tunnel 3). The tunnels are square concrete culverts 1-m wide and 1-m high, with respective lengths of 21 m (Tunnel 1), 40 m (Tunnel 2), and 18.5 m (Tunnel 3). Tunnels 1 and 2 are "dry fauna crossing structures," while Tunnel 3 is a hydraulic structure that had a slight trickle of water during the period of the experiments.

Capture and marking of specimens

Specimens of three amphibian species were captured using several methods depending on the species (**Table 1**): fire salamanders (*S. salamandra*) were collected on land when migrating to aquatic sites (from February to the beginning of April 2018 and 2019) during nocturnal transects of about 100 m

TABLE 1 Number of individuals of the three species tested in the different experiments.

		Without acoustic enrichment			**With acoustic enrichment**			**Total**		
Species	**Life stage**	**Tunnel 1**	**Tunnel 2**	**Tunnel 3**	**Tunnel 1**	**Tunnel 2**	**Tunnel 3**	**Tunnel 1**	**Tunnel 2**	**Tunnel 3**
Salamandra salamandra (1)	A	69	32					69	32	
Pelophylax kl. (2)	A			228			35			263
	J			61						61
Triturus cristatus (3)	A	229	59	62	46	46	71	275	105	133
	J	16	3	7	14	3	4	30	6	11

Life stage A, adult; J, juvenile. (1) Caught on land before reproduction; (2) caught in water before egg laying; (3) caught in water during reproduction.

along the HSR fences surrounding the Tunnel 2. A total of 101 individuals were caught and used for the experiments (**Table 1**). Water frogs (*Pelophylax* kl. *esculentus/lessonae)* (consisting of the edible frog *P. esculentus* and the pool frog *Pelophylax lessonae,* hereafter called *P. esculentus*) were caught in ponds (from 35 to 460 m from Tunnel 3) at the beginning of the breeding season (mid-May 2018–2019), manually or with a dip-net at night with the help of flashlight. A total of 324 individuals were caught and used for the experiments (**Table 1**). Great crested newts (*T. cristatus*) were caught in ponds during the reproductive period (from mid-February to mid-May 2018 and 2019) with minnow traps. A total of 560 individuals were caught and used for the experiments (**Table 1**).

Specimens were marked with transponders (Testud et al., 2020), and stored in opaque plastic tanks (0.80 × 0.40 × 0.25 m) with moist forest litter, kept outside, close to the capture site, in a shaded place with an ambient temperature ranging from 5°C (night) to 15°C (day) before the experiments. They were released at the exact place of capture (on land or pond) the day after the experiment. Authorization to catch amphibians was provided by the regional government of the study area (Regional Environment, Housing and Planning Agency of Sarthe, Mayenne, and Ille-et-Vilaine, inter-regional derogation from the prohibition to capture and transport specimens of protected animal species, 29 May 2017).

Design of the monitoring system and experiments

A monitoring system was designed, composed of four antennas (Biolog-id outdoor antennas, 100 × 8.5 × 2.5 cm), connected to an electronic control unit, described in detail in Testud et al. (2019). Each antenna included an auto-tuning function and a rechargeable power battery. With the PIT tag used, the detection distance of an antenna was 3 cm. The minimum time between the detection of two successive PIT tags was 70 ms. The antennae were equidistantly spaced (between each antenna and each end of the tunnel), delimiting five sections of the same length. For example, in the 40 m tunnel, the antennas were set up every 8 m (i.e., 8, 16, 24, and 32 m) from the entrance. Wooden reflectors were attached to each end of the antennas to prevent animals from avoiding crossing the antennas (**Figure 1B**).

Each individual was identified by a hand-recorder (reader RS1-F1, Biolog-ID©, reading distance = 4 to 10 cm). The individuals were released at the entrance every 1 min and the RFID antennas in the tunnel recorded each PIT-tagged individual with the precise time of crossing. Each individual was used only for one experiment.

These experiments were based on the concept of homing behavior, i.e., the propensity of displaced amphibians to come-back to the place of capture (Dole, 1972; Sinsch, 1987). *S. salamandra* were captured on land while migrating to a breeding place. The newt (*T. cristatus),* and the water frog (*P. esculentus*) were caught in ponds at dates when they were reproductively active. Newts caught in ponds can successfully return to the site of capture from displacement distances of more than 100 m (e.g., Twitty et al., 1964; Joly and Miaud, 1989). In our experiments, we performed only the first phase of the "homing" experiment design, i.e., the capture of individuals and their release at a place different from the capture place (the entrance of the tunnel). We expected that individuals will move to the capture place, located on the other side of the tunnel and then entering it.

Experiment was conducted for 22 nights from March to May 2018 and from February to May 2019. The experiments were performed after sunset (from approximately 7 p.m. to 9:30 p.m. from February to May). The experiment was stopped 4 h 30 min after the release of the last tested individual.

Evaluation of antenna effectiveness

When an animal completely crossed the tunnel, it was not necessarily detected by each antenna. The effectiveness of the antennas was evaluated as the proportion of animals detected by each antenna among the animals that completely crossed the tunnels. This was evaluated in 2019 with 125 adult *T. cristatus* and 22 *S. salamandra* in Tunnel 1, and 34 adult *T. cristatus*, and 53 *P. esculentus* in Tunnel 3.

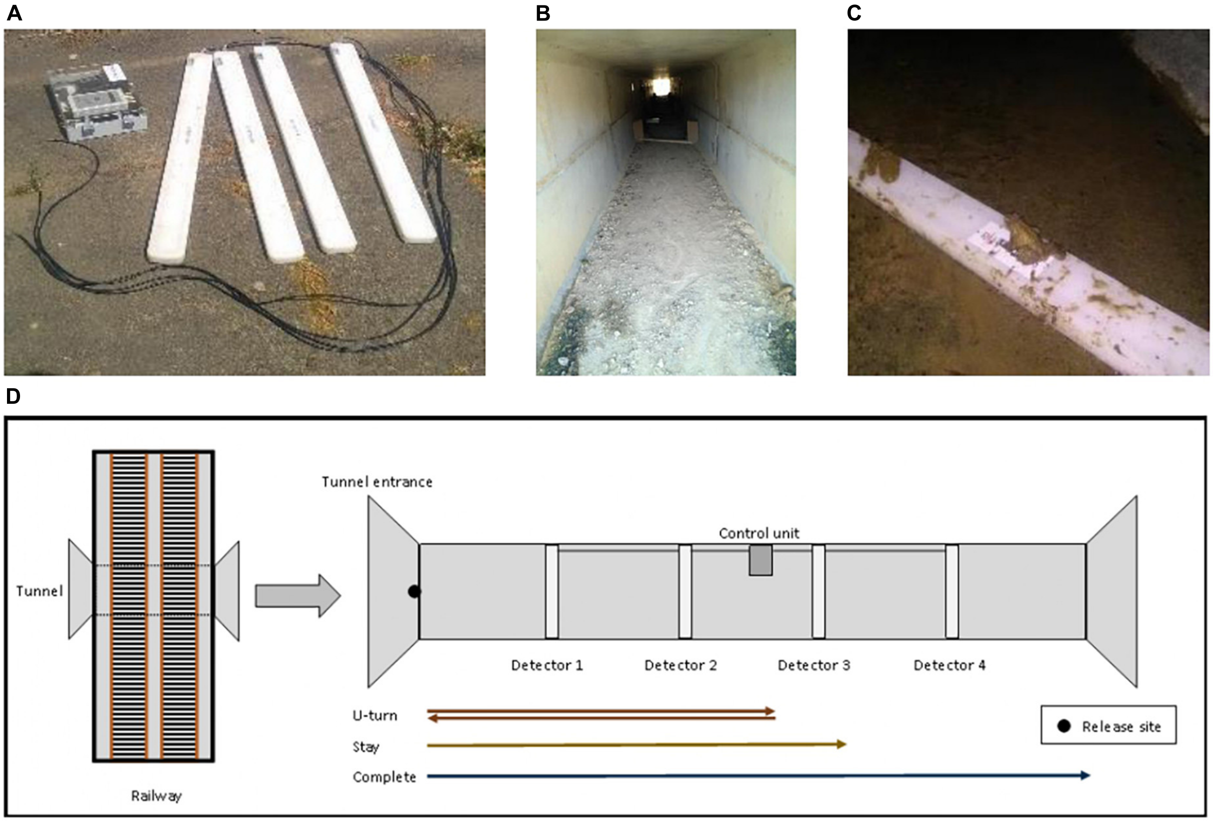

FIGURE 1
(A) The four antennas of the RFID device implemented in tunnels. **(B)** Internal view of one equipped tunnel. Wooden reflectors, set up on either end of one antenna, are visible. **(C)** A spined toad crossing one antenna. **(D)** Schematic view of the experiment. U-turn, Stay, and Complete are the behavioral categories recorded in the equipped tunnels.

Recording of individual trajectories, direction changes, and speed variation

The four RFID antennas installed in each tunnel recorded the successive positions of the animals, and the time series of these positions allowed the one-dimensional trajectory of each animal to be reconstructed. Individual trajectories and speed variation between tunnel sections were evaluated with individuals that performed a "complete crossing" (i.e., that completely crossed the tunnel) or performed a "U-turn" (i.e., that partly crossed the tunnel and then turned around and exited from the entrance). The maximum distance crossed in the tunnel (i.e., the farthest antenna from the entrance) was measured for each individual making a U-turn.

Acoustic enrichment

The acoustic enrichment experiment was performed in 2019 with a soundtrack of a mixed chorus of water frog (*P. esculentus*) and European tree frog (*H. arborea*), the two main species using mating calls in the region's amphibian community. The signals used for playback, the amplitude and the loudspeaker characteristics are provided in Testud et al. (2020). The stereo soundtrack was emitted using an audio player connected to two loudspeakers. These loudspeakers were installed outside the tunnel, 10 m from the exit and space 5 m apart. The soundtrack started when the first specimen was deposited at the tunnel entrance, and was stopped when the last released individual was collected. Great crested newt ($n = 184$) and water frog ($n = 35$) were tested in the tunnels with acoustic enrichment (**Table 1**).

Multi-state capture–recapture models

We constructed multi-state capture–recapture models (Lebreton et al., 2009) using the program E-SURGE v2.2.3 (Choquet et al., 2009). This approach allowed us to take advantage of the Markovian structure of multi-state models to first estimate the probability of an individual going forward or backward at each antenna in a tunnel and then evaluate whether acoustic enrichment increased the probability of going forward, depending on the position in the tunnel.

The successive positions of a marked specimen in the tunnel were described as a "state." The starting position in the tunnel for all individuals was defined as the state "Start." The antennas were named as states 1, 2, 3, and 4 according to their antenna number ("A1," "A2," "A3," and "A4"). The exit from a tunnel after a complete crossing ("CC") was named state 5, and the exit from a tunnel through the entrance after a U-turn ("UT") as state 6. The two matrices (of the initial state and of the probability of going forward are shown in **Supplementary Figure 1**). Individuals all began in state "Start," and then the transition matrix modeled the probability of moving forward to the next antenna, or going backward to the previous one (**Supplementary Figure 1**). The animals that stayed 10 min at the tunnel entrance without entering, and the animals that left the tunnel entrance without entering were not taken into consideration. Individuals that stopped in the middle of the tunnel were not considered. On the other hand, the animals that have walked a small distance without passing the first antenna before making a U-turn were used in the analysis. The total number of individuals used in the analysis was 293 for *T. cristatus* and 167 for *P. esculentus* (**Table 2**).

Using previous knowledge on antenna effectiveness, we chose to complete capture histories of individuals for which some detections were missing. The missing observations (event) were added to the detection history when the individual was detected with the previous and following antenna (an individual detected at antenna 1, then at antennas 3 and 4, and finally at the end of the tunnel would have the history: "start-1-2-3-4-5"). Individuals that exited through the entrance would have "start-6."

Model selection used a sequential backward procedure in which all candidate models were nested within the starting model. In the first step, we identified the most parsimonious structure for describing variation in going forward (or advancing) between antennas, and then the potential effect of acoustic enrichment. Exploratory analysis showed that the probability of going forward increased gradually as individuals advanced through the tunnel. Consequently, we tested for a linear response on the logit scale to summarize the responses with more parsimony, using only two parameters (intercept and slope)." The most parsimonious model was selected using the second-order Akaike Information Criterion (QAIC; Thomson et al., 2008; Choquet et al., 2009). Models with a difference of less than two QAIC units were considered equivalent in their ability to describe the data (Burnham and Anderson, 2002). To compare the significance of the best model with regard to the question of acoustic enrichment, the likelihood ratio test was calculated between the best model and its equivalent with or without enrichment. Four datasets were analyzed, three datasets for *T. cristatus* (three tunnels), and one dataset for *P. esculentus* (**Table 2**).

Results

Antenna effectiveness

The effectiveness of the antennas (proportion of animals detected by the antennae of the animals that completely crossed the tunnels) varied, mainly according to species (**Table 3**). Antenna effectiveness in Tunnel 1 and Tunnel 3 was rather similar for the two Urodela species (*T. cristatus* and *S. salamandra*), varying from 0.68 ± 0.16 to 0.97 ± 0.06. Both *S. salamandra* and *T. cristatus* marked individuals were detected by at least one antenna (except one newt) (**Figure 2**). For the Anuran species *P. esculentus*, the effectiveness varied from 0.08 ± 0.07 to 0.38 ± 0.13 (**Table 3**), with lower detections at the two first antennas. For *P. esculentus* individuals, 42% of the marked specimens were not detected by at least one antenna (**Figure 2**).

General characteristics of trajectories in the tunnels

Several types of movement were detected during a complete crossing: a rather "linear" trajectory at constant

TABLE 2 Number of individuals making a "complete crossing" or a "U-turn," with and without acoustic enrichment, for the four datasets analyzed using multi-state models.

	T. cristatus						***Pelophylax* kl.**	
	Tunnel 1 (21 m) 8, 10, 17, and 18 April 2019		**Tunnel 2 (40 m) 20 and 21 May 2019**		**Tunnel 3 (18.5 m) 15 and 16 May 2019**		**Tunnel 3 (18.5 m) 15 and 16 May 2019**	
	Complete crossing	**U-turn**	**Complete crossing**	**U-turn**	**Complete crossing**	**U-turn**	**Complete crossing**	**U-turn**
With enrichment	61	61	7	33	15	35	32	2
Without enrichment	48	47	7	29	19	32	21	11

TABLE 3 Effectiveness of the antennas to detect marked individuals of the three amphibian species in Tunnel 1 (21 m in length) and Tunnel 3 (18.5 m in length).

	Antenna 1	Antenna 2	Antenna 3	Antenna 4
Salamandra salamandra (*N* = 22) in Tunnel 1	0.73 ± 0.19	0.77 ± 0.18	0.91 ± 0.12	0.77 ± 0.18
Triturus cristatus (*N* = 125) in Tunnel 1	0.74 ± 0.08	0.80 ± 0.07	0.84 ± 0.06	0.94 ± 0.04
Triturus cristatus (*N* = 34) in Tunnel 3	0.85 ± 0.12	0.68 ± 0.16	0.97 ± 0.06	0.94 ± 0.08
Pelophylax kl. (*N* = 53) in Tunnel 3	0.08 ± 0.07	0.11 ± 0.09	0.38 ± 0.13	0.36 ± 0.13

Effectiveness is the proportion of animals detected by the antenna among the animals that completely crossed the tunnels (expressed in % with confidence intervals of 95%).

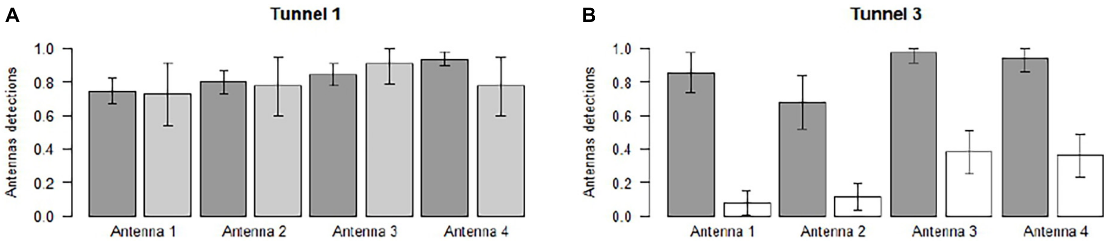

FIGURE 2
Effectiveness of the antennas correspond to the proportion of animals detected by the antenna among the animals that completely crossed the tunnels. Antenna effectiveness was measured in Tunnel 1 **(A)** and Tunnel 3 **(B)**. Dark gray bars represent *T. cristatus*, light gray bars represent *S. salamandra*, and white bars represent *Pelophylax Kl.*

speed (Figures 3A,B) or non-linear trajectories (Figures 3C–I). Movements with varying speeds were also observed (the animal might accelerate or slowdown in the tunnels). These behaviors are visible in Figures 3C,D when a trajectory “stalls” as compared to an average constant speed estimated from the crossing duration. Individual trajectories can be characterized by a “halt,” when the amphibian was detected several times by the same antenna (Figures 3E,F), and by “back-and-forth” movements (Figures 3G,H). These types of trajectories could be present alone (Figure 3F) or consecutively (Figure 3I).

Similar types of movements were observed for animals that made a U-turn (Supplementary Figure 2): for example, back-and-forth trajectories (Supplementary Figure 2i). The farthest antenna from the entrance that the amphibian crossed is also available (e.g., First antenna: Supplementary Figure 2g; fourth antenna: Supplementary Figure 2b).

Individual trajectories in tunnels of varying length for *Salamandra salamandra* and *Triturus cristatus*

In *S. salamandra*, the trajectories were recorded in Tunnel 1 (21 m) and Tunnel 2 (40 m) (Supplementary Figure 3). In Tunnel 1, among the individuals that made a complete crossing, the trajectories of 16 salamanders were available (Supplementary Figure 3a), most had a linear trajectory, and some back-and-forth movements were observed. One individual was strongly distinguished from the others by its long crossing time and back-and-forth travel. One individual halted at antenna 1 for more than 3 h, before quickly crossing the rest of the tunnel. Among the individuals that made a U-turn (Supplementary Figure 3b): 1 individual made a rapid U-turn after crossing the first antenna at 4 min 20 s, 1 made a 3-h stop near the same antenna and 1 made a back-and-forth movement twice, getting closer to the exit before finally making a U-turn. In Tunnel 2 (40 m), among the individuals that made a complete crossing, the trajectories of nine salamanders were available (Supplementary Figure 3c), and most of the individuals moved with linear trajectories. No back-and-forth movements were observed. Among the individuals that made a U-turn, the trajectories of four salamanders were available (Supplementary Figure 3d), one individual went 8 m before turning around, two reached antenna 2 (16 m) before turning around, and one reached antenna 3 (24 m), where it stayed for about 1 h before making a U-turn.

In *T. cristatus*, the trajectories were recorded in Tunnel 1 and Tunnel 2 (Figure 4). Among the individuals that made a complete crossing, the trajectories of 73 newts were available

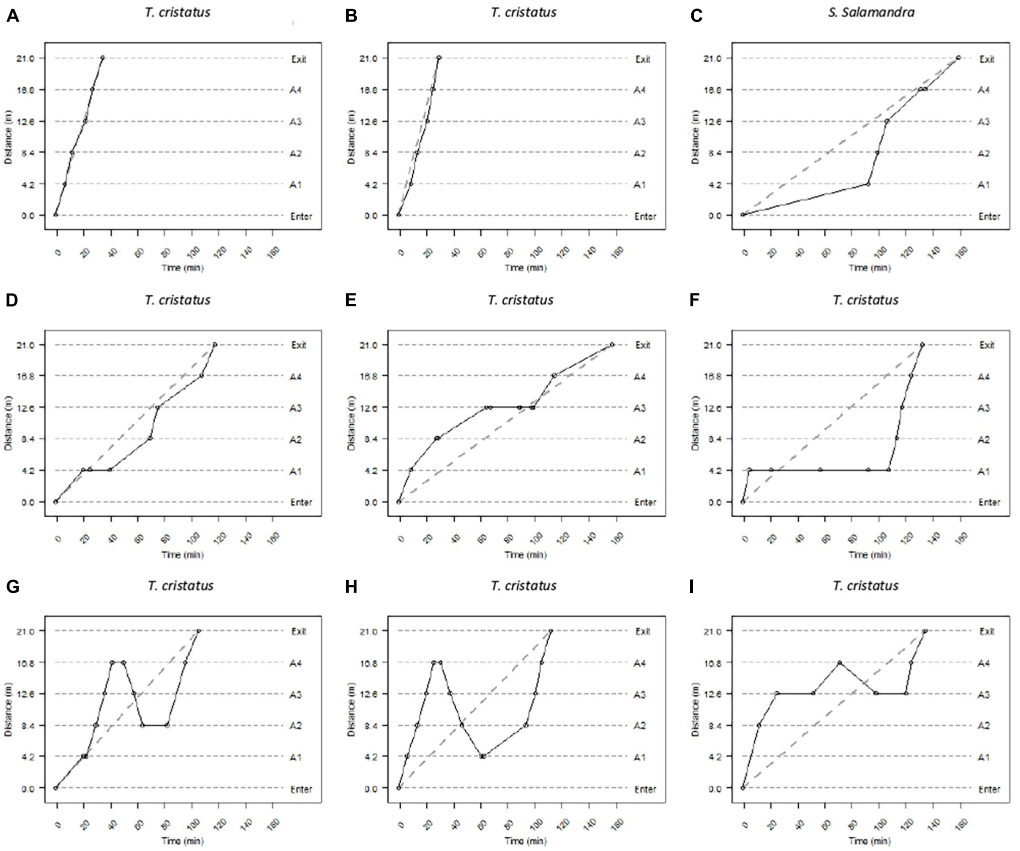

FIGURE 3
Different types of movement exhibited during a complete crossing. The dashed lines represent the entry, exit, and location of the antennas in the tunnels. The positions of the individuals in the tunnels at the time since release are represented by dots. The solid lines represent the speed of movement between the different positions. Panels **(A–I)** represent several patterns of movement (see section "Results").

(Figure 4A), with a linear trajectory with a fast or slow constant speed, a non-constant speed, a halt, or back-and-forth movements. In Tunnel 2 (40 m), among the individuals that made a complete crossing, the trajectories of nine newts were available (Figure 4E), and six had a linear trajectory, including five at a fast speed and one at a slow speed. One individual had a non-constant speed. Among the individuals that made a U-turn, the trajectories of 38 newts were available in Tunnel 1 (Figure 4B). Four individuals reached the maximum distance of 16.2 m (antenna 4), two individuals halted and exhibited back-and-forth behavior. In Tunnel 2, among the individuals that made a U-turn, the trajectories of 20 newts were available (Figure 4F), and 2 individuals reached the maximum distance of 16 m (antenna 2).

The maximum distance traveled in Tunnel 1 (21 m) before turning back was 16.8 m (antenna 4) for the salamander and the newts. In Tunnel 2 (40 m), this maximum distance was 24 m for the salamander (antenna 3) and 16 m for the newts (antenna 2). For this species, the probability of going forward from "Start" to antenna 2 in Tunnel 1 was $\Psi_{\text{Start/Tunnel 1}} \times \Psi_{\text{A1/Tunnel 1}} = 0.56$, and was $\Psi_{\text{Start/Tunnel 2}} = 0.39$ in Tunnel 2. This higher propensity was found in the next 8 m with $\Psi_{\text{A2/Tunnel 1}} \times \Psi_{\text{A3/Tunnel 1}} = 0.75$, and was ($\Psi_{\text{A1/Tunnel 2}} = 0.46$) in Tunnel 2.

Individual trajectories in tunnels with acoustic enrichment for *Triturus cristatus* and *Pelophylax esculentus*

In *T. cristatus*, individual trajectories were recorded in Tunnel 1 and Tunnel 2 with acoustic enrichment (Figure 4). The types of trajectory of the 32 newts performing a complete crossing in Tunnel 1 (Figure 4C, 53%) were similar to the trajectory observed without enrichment (Figure 4A). Six individuals performed a U-turn (Figure 4D, 10%), and 1 turned around after reaching 16.8 m (antenna 4). Two individuals halted at 4.20 m (antenna 1) for about an hour. In Tunnel 2, five newts made a complete crossing (Figure 4G, 10%), four individuals had a non-constant speed and one halted. Nine newts performed a U-turn (Figure 4H, 18%), and one individual turned around after reaching 32 m (antenna 4).

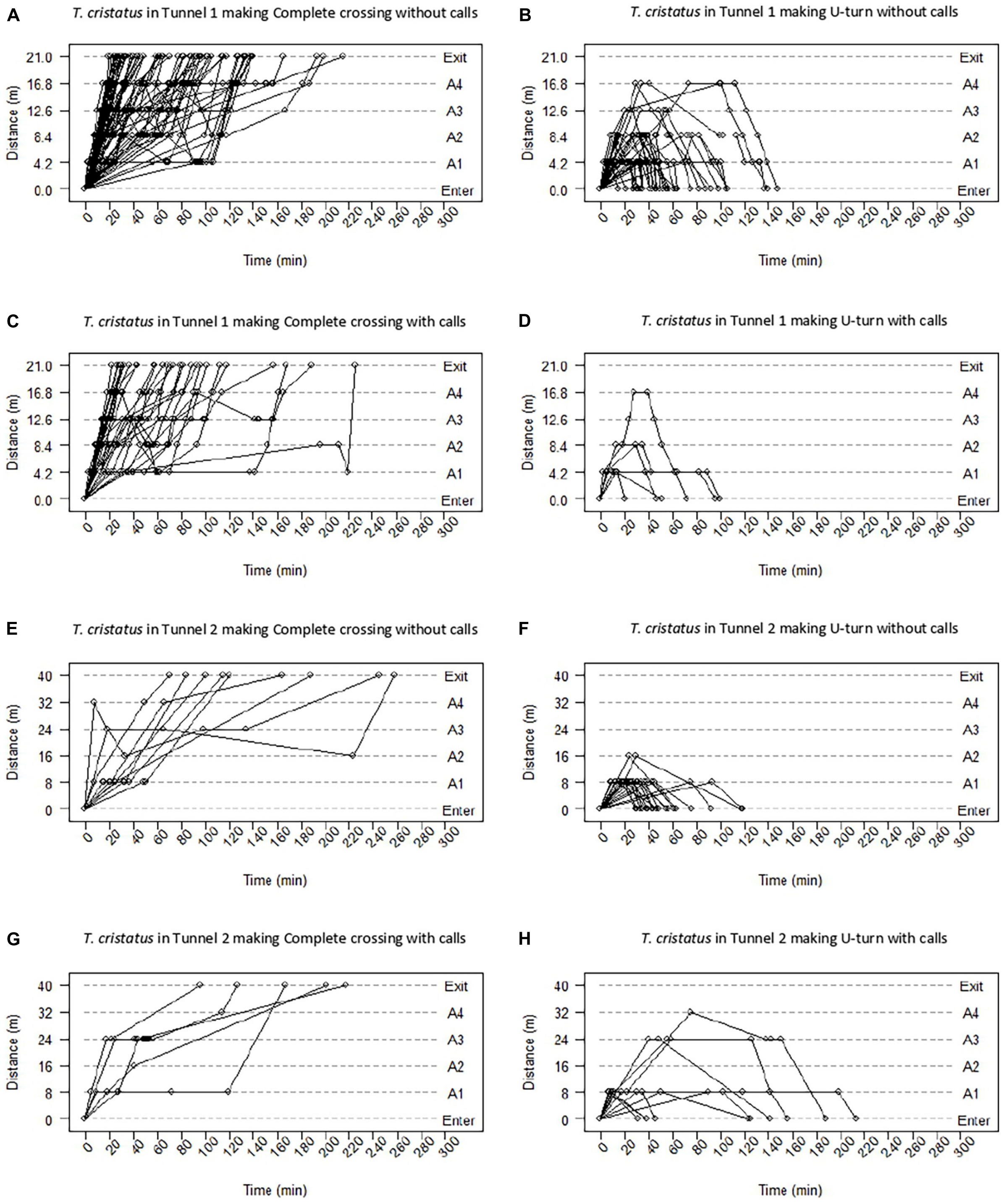

FIGURE 4
Trajectories of *T. cristatus* in Tunnels 1 and 2 without acoustic enrichment (T1) and with acoustic enrichment (T2) to complete crossing (left) and U-turn (right). The dashed lines represent the entry, exit, and location of the antennas in the tunnels. The positions of the individuals in the tunnels at the time since release are represented by dots. The solid lines represent the speed of movement between the different positions. Panel **(A)** represents trajectories of newts making a complete crossing in Tunnel 1 without enrichment. Panel **(B)** represents trajectories of newts making a U-turn in Tunnel 1 without enrichment. Panel **(C)** represents trajectories of newts making a complete crossing in Tunnel 1 with enrichment. Panel **(D)** represents trajectories of newts making a U-turn in Tunnel 1 with enrichment. Panel **(E)** represents trajectories of newts making a complete crossing in Tunnel 2 without enrichment. Panel **(F)** represents trajectories of newts making a U-turn in Tunnel 2 without enrichment. Panel **(G)** represents trajectories of newts making a complete crossing in Tunnel 2 with enrichment. Panel **(H)** represents trajectories of newts making a U-turn in Tunnel 2 with enrichment.

In *P. esculentus*, individual trajectories were recorded in Tunnel 3 (18.5 m), with and without acoustic enrichment conditions (**Supplementary Figure 4**). Without enrichment, 53 frogs performed a complete crossing (**Supplementary Figure 4a**, 18%), the types of movement were a linear trajectory with a fast or slow constant speed, non-constant speed, a halt, or back-and-forth movement. The same types of movements were observed in the 18 frogs that made a U-turn (**Supplementary Figure 4b**, 6%). One individual reached the maximum distance of 14.8 m (antenna 4). With enrichment, 16 frogs performed a complete crossing (**Supplementary Figure 4c**, 46%) and exhibited mainly fast linear trajectories. Only one individual did a U-turn, after reaching 3.7 m (antenna 1) (**Supplementary Figure 4d**).

Modeling probability of going forward with and without enrichment

The five best models from the selection procedure are shown in **Supplementary Table 1**. For *T. cristatus* in Tunnel 1, the best model included a linear relationship between all four antennas, as well as significant effects of acoustic enrichment on both the slope of the linear relationship and the probability of going forward from the "Start" state (**Figure 5A**), for this model the LRT provides a *p*-value of 0.0333. With enrichment, the probability of going forward from "Start" ($\Psi_{Start/With}$ = 0.78, 95% CI: 0.70–0.84) was 18% higher than without enrichment ($\Psi_{Start/Without}$ = 0.66, 0.56–0.75; ΔQAIC = 2.77). The probability of going forward increased as the individual advanced in the tunnel; this probability was 27% higher at antenna 4 than at antenna 1. The lower probability of advancing with enrichment observed at antenna 1 ($\Psi_{A1/Without}$ = 0.80, 0.70–0.86; $\Psi_{A1/With}$ = 0.69, 0.61–0.77) is most likely due to the fact that more individuals passed the first antenna. Of these individuals, several turned back after having explored more of the tunnel, thus decreasing the probability of going forward at antennas 1, 2, and 3.

For *T. cristatus* tested in Tunnel 2, the best model from the selection procedure included a linear relationship from antenna 2 to antenna 4, with a significant effect of acoustic enrichment on its slope (**Figure 5B**), for this model the LRT provides a

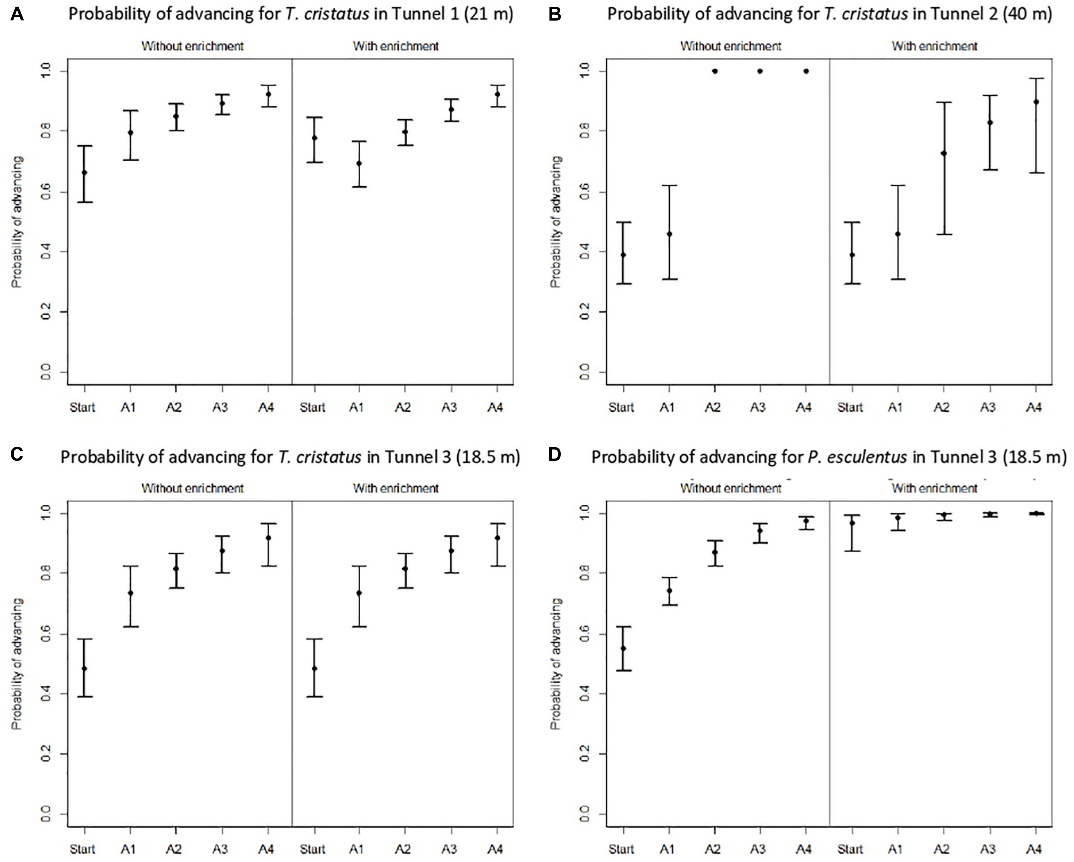

FIGURE 5
Probability of going forward (advancing) for the great crested newt in the three tunnels **(A–C)**, and the water frog in Tunnel 3 **(D)**. Mean probability and confidence intervals of 95% were estimated with the best model from the selection procedure.

p-value of 0.02011. The probabilities of going forward at "Start" and at antenna 1 were different (Ψ_{Start} = 0.39, 0.29–0.50 to Ψ_{A1} = 0.46, 0.31–0.62), but comparable with or without acoustic enrichment. No individuals turned back beyond antennas 2, 3, and 4 in the condition without enrichment [the slope of the linear relationship (**Figure 5B**) is equal to 1]. In the condition with enrichment, individuals turned back at a further distance in the tunnel. The acoustic signal to attract individuals could be limited to a distance between 24 and 32 m, as evidenced by the lack of effect of enrichment at the start and at antenna 1.

For *T. cristatus* tested in Tunnel 3, the best model was the one with a linear constraint between the five positions and no significant effect of acoustic enrichment (ΔQAIC = 1.15), for this model the LRT provides a p-value of 0.3451. It thus had two parameters: the intercept and the slope of the line, with a gradual increase of the probability of going forward as a function of location in the tunnel from Ψ_{Start} = 0.51, 0.43–0.60 to Ψ_{A4} = 0.94, 0.88–0.97 (**Figure 5C**).

The last dataset analyzed corresponds to *P. esculentus* tested in Tunnel 3. The model with a linear line between the five positions with a significant effect of acoustic enrichment on the intercept was the best model from the selection procedure (ΔQAIC = 46.39), for this model the LRT provides a p-value of 3.501e−12. There was a gradual increase in the probability of going forward as a function of location in the tunnel, from $\Psi_{\text{Start/Without}}$ = 0.55, 0.48–0.62 to 4, $\Psi_{A4/\text{Without}}$ = 0.97, 0.94–0.99 (**Figure 5D**). The model supported a strong effect of acoustic enrichment with the probability of going forward at "Start" ($\Psi_{\text{Start/With}}$ = 0.97, 0.87–0.99): 78% higher than without enrichment and to a lesser degree at the antennas.

Discussion

PIT tags and RFID antennas in wildlife passages

The lack of behavioral data on small species crossing wildlife passages is partly due to methodological issues (Weber et al., 2019); evaluating the behavior of amphibians in tunnels is challenging (review in Testud et al., 2019). Marking amphibians with fluorescent pigments to record continuous tracks in tunnels has been used, but the number of monitored individuals is limited and does not provide information on data such as movement speed (Matos et al., 2018). Camera traps are also widely used for movement tracking in tunnels (reviewed in Jumeau, 2017). While their detection rate has been reported as low for amphibians (Pagnucco et al., 2012), the increase in the quality of video traps implemented makes them more relevant for such small species (Hobbs and Brehme, 2017; Pomezanski and Bennett, 2018; Jarvis et al., 2019; Brehme et al., 2021). Using PIT tags is a traditional marking technique in amphibians (e.g., Perret and Joly, 2002; Winandy and Denoël, 2011; Testud et al., 2019; Weber et al., 2019). Telemetry using RFID antennas is employed to monitor movement patterns and habitat use in small animals (Charney et al., 2009), to evaluate fish passes (Thiem et al., 2011, 2013; Ovidio et al., 2017; Benitez et al., 2018; Lothian et al., 2019) and tunnels (Boarman et al., 1998). The use of RFID antennas to track amphibian movements and habitat use in nature is increasing (Winandy and Denoël, 2011; Atkinson-Adams, 2015; Atkinson-Adams et al., 2016; Testud et al., 2019; Weber et al., 2019). To our knowledge, to date, PIT-tagged amphibians and RFID antennas have been used to evaluate equipped wildlife passages for only one toad and two carabid species (Testud et al., 2019). This design was also used to compare the performance of this type of monitoring with camera traps in tunnels crossed by a salamander species (Atkinson-Adams, 2015).

Our experiments with a system composed of four RFID antennas improve knowledge on how amphibians move inside wildlife crossings. The effectiveness of the system varied between antennas and species. The locomotion of species can greatly differ (e.g., exclusively walking locomotion in Urodela, exclusively saltatory locomotion in frogs, or both in toads). In our experiments, the lower detection rate observed with frogs (**Figure 2** and **Table 2**) may be explained by their jumping over the antenna, the maximum detection distance of the antenna being 3 cm. The detection range could be improved using another antenna design (e.g., circular), but with the practical constraint of implementing this in the tunnels. Higher but not total detection rates were observed with the salamander and newt species (**Figure 2** and **Table 2**). The antennas were 8.5 cm in width (**Figure 1**), and two marked specimens crossing the antennas simultaneously stop the recording. If one individual stays on the antenna, it will prevent the recording of the other individual crossing during this time. Thinner antennas and better positioning on the tunnel floor (i.e., not disturbing the floor) may increase the detection rate. Camera traps detected 44% of crossings of the long-toed salamander (*Ambystoma macrodactylum*), producing a large number of empty images (Pagnucco et al., 2011). By installing an RFID antenna at the exit of a tunnel, the detectability of the camera traps was reevaluated to a lower 15.3% (Atkinson-Adams, 2015). These kinds of comparative studies are valuable in the context of amphibian-crossing tunnels.

Amphibian behavior to evaluate wildlife passages

Tunnel effectiveness can be evaluated with the crossing rate (proportion of animals that cross the tunnels or not) and the mean speed of crossing. These parameters are used to evaluate tunnel characteristics such as length and diameter (Patrick et al., 2010; Testud et al., 2020), substrate (Lesbarrères et al., 2004; Trochet et al., 2019), light, humidity and temperature (Bain, 2014; Bain et al., 2017) and social information, including acoustic enrichment (Testud et al., 2020). However, the same duration in a tunnel may involve very different movement types, and more information about the behavior inside tunnels is needed to improve the evaluation of tunnel effectiveness (Matos et al., 2018). We observed a variety of movements with Urodela species in tunnels (**Figures 3**, **4**): some individuals crossed the tunnels with a linear trajectory, while others made back-and-forth movements. Some individuals halted their movement in different parts of the tunnel, for different amounts of time.

The maximum distance traveled in tunnels could be an indicator of crossing effectiveness, especially in long tunnels (e.g., HSR and Highways). Individuals performing a U-turn can be also very informative for evaluating tunnel characteristics, as no U-turn by newts or salamanders was observed farther than 16 and 24 m respectively in the 40-m tunnel. In all cases, the probability of going forward increased as the amphibian progressed in the tunnel (**Figure 5**). The distance before making a U-turn and an increase in the probability of going forward should vary according to a threshold based on parameters linked to individual motivation (Joly, 2019), including abiotic factors (e.g., seasonality; Matos et al., 2018), biotic factors (species; Ovidio et al., 2017), personality (Chajma et al., 2020), individual history, or social conditions (including acoustic enrichment).

Individual trajectories to evaluate the effects of tunnel length

The length of a tunnel acts on the proportion of complete crossings (higher in *S. salamandra* and *T. cristatus* in the shorter tunnel; Testud et al., 2020), as previously shown in several amphibian species (Dodd et al., 2004; Lesbarrères et al., 2004; Patrick et al., 2010). Considering the precise movements in a tunnel (this study), the propensity to go forward in the first meters of the tunnel (8 m) was higher for the shorter tunnel. These results argue for experiments that explore the mechanisms of newts' appreciation of tunnel length: e.g., perception of the exit (odor, brightness, temperature, ventilation, etc.) or distance traveled (Brehme et al., 2021).

Individual trajectories to evaluate acoustic enrichment

Acoustic enrichment to lure animals to specific places is a new tool for active management on land and at sea (Gordon et al., 2019; Putman and Blumstein, 2019). In the terrestrial environment, this has been shown to be effective in birds and mammals (Ward and Schlossberg, 2004; Kiffner et al., 2008; Molles et al., 2008; Friesen et al., 2017). In amphibians, acoustic enrichment with conspecific or heterospecific calls can improve the colonization of new ponds (Buxton et al., 2015; James et al., 2015) and can attract newts (Diego-Rasilla and Luengo, 2004, 2007; Pupin et al., 2007; Madden and Jehle, 2017). When a soundtrack of mating calls of syntopic anurans was broadcast in a tunnel (Testud et al., 2020), frogs exhibited a large increase in complete crossings and speed, and newts showed an increase in complete crossings (but not speed) in one of the acoustically enhanced tunnels.

In this study, recording the individual trajectories improved the evaluation of the effect of acoustic enrichment. In the acoustically enriched tunnel, water frogs exhibited more linear movements, and more newts exhibited back-and-forth movements. Newts also made a U-turn at a farther distance (32 m) in the acoustically enriched tunnel. We posit that acoustic enrichment could modify the behavior of novice or hesitant individuals and shift the distance that discourages these individuals from turning back.

Acoustic enrichment had an effect on the probability of going forward for newts (in Tunnels 1 and 2, but not in Tunnel 3). In Tunnel 1, with enrichment, an increase in the probability of going forward at "Start" and then a decrease at the next antenna can be explained by the increase in the number of back-and-forth movements. In Tunnel 2, with enrichment, the effect of enrichment on the probability of going forward was only observed in the second half of the tunnel. The role of the distance to the sound source on the propensity to go forward in the tunnel remains to be confirmed.

Acoustic enrichment had an effect on the probability of going forward for frogs in Tunnel 3, where the probability reached values close to 1, and result for newts in Tunnels 1 and 2 attesting to the effectiveness of the use of amphibian calls to improve passage in tunnels. These results support the hypothesis that acoustic enrichment makes wildlife crossings more attractive (Gerhardt and Huber, 2002), and improves tunnel crossings by amphibians (Testud et al., 2020).

Conclusion

The use of an RFID multi-antenna system can provide information on how small animals behave when crossing tunnels (Testud et al., 2019), thus allowing tunnel characteristics to be tested to improve their use (Pomezanski and Bennett, 2018; Testud et al., 2019). However, the price of the device (reader and antenna) and the detection distance of marked individuals could be limiting (Winandy and Denoël, 2011; Testud et al., 2019; Weber et al., 2019). The simultaneous use of different methods, e.g., multiple fluorescent pigments, camera traps and RFID systems, in different contexts and with different species, would be a useful way to compare parameters such as individual detection rate (Eggert, 2002; Atkinson-Adams, 2015; Bain et al., 2017; Hobbs and Brehme, 2017; Matos et al., 2018; Testud and Miaud, 2018; Jarvis et al., 2019).

There is a high variability in wildlife passage crossing success in Amphibians (Brehm, 1989; Allaback and Laabs, 2003; Dodd et al., 2004; Lesbarrères et al., 2004; Woltz et al., 2008; Patrick et al., 2010; Malt, 2011; Hamer et al., 2014; Bain et al., 2017; Matos et al., 2018; Pomezanski and Bennett, 2018;

Jarvis et al., 2019; Chajma et al., 2020). This variability can come from individual differences (this study), which has to be considered as behaviors (including movement) at the scale of the individual can have repercussions for the functioning of an entire ecosystem (e.g., Bowler and Benton, 2005; Allgeier et al., 2020).

There is also a growing need to evaluate amphibian behavior in wildlife passages (and not only their use of these crossings), and methodological advances such as RFID and other techniques that allow fine tracking are promising. Experiments that consider physiological states of individuals, age, experience of migratory routes, etc., would all increase our understanding considerably. Acoustic behavior may influence species differently. Experiments to assess the effect of acoustic enrichment with other amphibian communities would also be valuable, to evaluate the possible generalization of the method used in this study. Wildlife passages such as under-road tunnels are often equipped with barrier fencing to reduce mortality and direct amphibians toward the passages. A better knowledge of animal movements along the fencing (proportion of animals passing along them, entering the tunnels, etc.) can also help to design effective LTI mitigation for amphibians (Brehme et al., 2021).

Methodological developments (e.g., RFID, Testud et al., 2019; camera traps, Brehme et al., 2021; Conan et al., 2022) allow designing experiments to test for tunnel permeability and its link with individual behaviors, e.g., tunnel attractiveness including surroundings of the entrance and local conditions within the tunnel (substrates, open grated top to allow for natural light, temperature and moisture conditions). Knowledge about the probability of crossing a structure is also particularly relevant for friction map modeling and species distribution modeling at a regional scale in landscapes, which are often fragmented by linear transport infrastructure (Remon et al., 2018; Clauzel and Godet, 2020; Matutini et al., 2021).

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Structure Chargée du Bien Etre Animal, CEFE, Montpellier.

Author contributions

GT, CM, TL, and DL participated in the design of the experiments. GT and CC did the statistical analysis. QL, GT, and DP did the field experiments. GT and CM wrote the manuscript. All authors reviewed the manuscript.

Funding

This work was supported by the French National Research Agency (ANR), the French engineering group Egis (CIFRE grant number 2017/0212), and the French construction group Eiffage.

Acknowledgments

We would like to thank Marie-Aurélia Sabatte, Eva Garcia, Agathe Legrand, Anthony Martin, Camille Clowez, Philippe Geniez, Emelie Segrestan, Lynda Farineau, Annick Lucas, François Le Hérissé, Julien Maziere, Margot Piccinin, Clement Fauconnier, and Adrien Parais for their field participation. We are also grateful to the Janvier and to Fred and Arnaud for their hospitality. Philippe Cordier (Biolog-id©) offered us valuable RFID assistance. We also thank the two reviewers who greatly help improving the first draft of this manuscript. We also thank Philippe Geniez, Aurélien Besnard, and Pierre-André Crochet for their advice as well as the reviewers and editors for the improvements made to this manuscript. The constructive discussions with Sandra Luque, David Lesbarreres, Françoise Burel, Alain Morand, and Sylvie Vanpeene-Bruhier also contributed to the realization of this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.958655/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 25 November 2022
DOI 10.3389/fevo.2022.944848

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 15 May 2022
ACCEPTED 08 November 2022
PUBLISHED 25 November 2022

CITATION
Wilkinson JA and Romansic JM (2022) The effect of road-based mortality on a local population of newts along a narrow two-lane road in California.
Front. Ecol. Evol. 10:944848.
doi: 10.3389/fevo.2022.944848

The effect of road-based mortality on a local population of newts along a narrow two-lane road in California

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Numerous studies have documented high numbers of amphibians killed by vehicular strikes on roads. This direct mortality can cause population declines and extirpations, but in some cases the declines might be masked, initially, by large population sizes. Population viability analysis can help discern population trajectories and identify incipient declines. We applied this tool to a situation in Santa Clara County, California where a dead-on-the-road carcass survey in 2017 demonstrated that a large number of newts in the genus *Taricha* were being killed by vehicles using a small two-lane road (Alma Bridge Road) most likely during annual breeding migrations to a local reservoir (Lexington Reservoir). To help determine the effect of this road-based mortality on the California newt (*T. torosa*) population, we conducted a drift fence/pitfall trap array study on the road during the 2020–2021 breeding season. Drift fence/pitfall trap arrays were installed at six locations along a 6.6-km stretch of the road and daily surveys were conducted at these arrays over a 148-day period from mid-November to end of March. Daily traffic and precipitation data were also recorded to help inform timing of proposed mitigation efforts. Concurrently, a group of community scientists conducted a dead-on-the-road carcass survey over the entire 6.6-km study area. We calculated the number of adult newts attempting to cross Alma Bridge Road at the arrays and the associated road-based mortality rates. Then, we combined our array results with road mortality data provided by the community scientists to estimate the number of adult California newts attempting to cross the road and their mortality rates over the entire study area during the survey period. We then incorporated this data into a population viability model to determine whether this road-based mortality rate might, if left unabated, lead to a reduction in, and possibly the eventual extirpation of, the local population of *T. torosa* breeding in Lexington Reservoir. The model indicated that this population would be extirpated in approximately 92 years. Because the road has been in use for approximately 67 years, we discussed the possible reasons why this population is currently extant and experiencing this high level of road-based mortality.

KEYWORDS

California newt, rough-skinned newt, road-based mortality, drift fence/pitfall trap array, carcass survey, population viability model

Introduction

Roads and traffic have long been known to have major impacts on animal populations and communities (Stoner, 1925; Trombulak and Frissell, 2000; Forman et al., 2003). One study estimated that one million vertebrates are killed each day on roads in the United States (Lalo, 1987), and another emphasized that road-kill is a factor in the overall decline of amphibians (Glista et al., 2007). The negative effects of roads and traffic on amphibian populations are well known (Beebee, 2013), and although road mortality may not have a substantial effect on very large populations, it can negatively affect populations of threatened or endangered species over time (Glista et al., 2007). For this reason, three threatened and endangered amphibian species with ranges in or near the San Francisco Bay Area in California have received much attention with respect to the negative impacts of roads: the California tiger salamander (*Ambystoma californiense*), which is listed under both the Federal and California Endangered Species Acts as threatened, with the Sonoma and Santa Barbara County populations listed federally as endangered (Bain et al., 2017); the Santa Cruz long-toed salamander (*A. macrodactytum croceum*), which is federally and state listed as endangered (Hobbs, 2013); and the California red-legged frog (*Rana draytonii*), which is federally listed as threatened, and is state listed as a species of special concern. Concern about potential impacts of roads on their populations has had some influence on how roads are currently constructed and used within the ranges of these species, with some road designs incorporating measures such as barriers to over-the-road movement coupled with undercrossings to allow amphibians to safely cross the roads (Bain et al., 2017). In some areas, compensatory mitigation is required to reduce the impacts of new road construction on these species through land conservation (e.g., U.S. Fish and Wildlife Service, 2005).

A study by Brehme et al. (2018) ranked species of amphibians and reptiles in California based on the risk of roads to their populations. Each species was given a road risk score based on movement distance, frequency of movement, habitat preference, road use, movement speed, fecundity, proportion of population at risk, size of range or amount of isolation, and conservation status, and then grouped into five broad categories of very high risk, high risk, medium risk, low risk, and very low risk. Of the 33 species in the very high risk category, eight were amphibians and 25 were reptiles. Of the eight amphibian species in this category, the top four were salamanders: red-bellied newt (*Taricha rivularis*) with a score of 561, California newt (*T. torosa*) with a score of 532, then California tiger salamander, and Sierra newt (*T. sierrae*), which both received a score of 437.

The placement of the California tiger salamander in the very high risk category is understandable, given its threatened and endangered status as mentioned above. However, the placement of the red-bellied newt and the California newt above, and the Sierra newt at the same level as, the California tiger salamander is less intuitively obvious, as the red-bellied newt and the California newt are listed only as California species of special concern (only the southern California populations of the California newt are listed as such), and the Sierra newt has no special listing status. Everything being equal, the California tiger salamander should have a higher risk factor than these other species because of its threatened and endangered status. These rankings reflect higher individual-level and population-level risk scores in the California newt and the Sierra newt due to the other factors in the risk model, such as longer movement distances and higher frequency of movement than other species, which would increase their chances of crossing (and encountering risk) on a road, along with evidence of high mortality on roads and early disappearance following landscape fragmentation (Brehme et al., 2018). Another interesting finding of Brehme et al. (2018) is the placement of the rough-skinned newt (*T. granulosa*) within the high risk category along with the Santa Cruz long-toed salamander, with road risk scores of 304 and 308, respectively. The rough-skinned newt has no federal or state protective status, and has a relatively large range of four or more states and four geographic regions within California, whereas the Santa Cruz long-toed salamander is restricted to the southern Santa Cruz and northern Monterey Counties (U.S. Fish and Wildlife Service, 2004). Again, these rankings reflect high individual-level and population-level risk scores.

Both the rough-skinned newt and the California newt inhabit the mountainous parts of the San Francisco Bay Area, but have been mostly extirpated from the lower elevations because of habitat loss caused by development. The distribution of the rough-skinned newt extends south along the San Francisco Peninsula to southern Santa Cruz and southwestern Santa Clara counties. On the other hand, the California newt has several disjunct distributions from Mendocino County south to southern California, one of which is along the San Francisco Peninsula similar to the rough-skinned newt; the California newt also occurs east of the bay in the hills and mountains of western Contra Costa and Alameda counties, south to southern Santa Clara and southwestern San Joaquin counties. Both the rough-skinned newt and California newt are found in sympatry within San Mateo, western Santa Clara, and Santa Cruz counties (Stebbins and McGinnis, 2012).

Even though these two species do not have state or federal protective status in the Bay Area, there is some recognition that development (including roads) is having an impact on their survivorship. Accordingly, some measures have been introduced to protect these species from road mortality. For example, each year the East Bay Regional Parks District closes South Park Drive in Tilden Park from November to March to protect California newts crossing the road during the winter rainy season from vehicle strikes[1], and volunteers in Marin assist newts in safely crossing Chilleno Valley Road.[2]

Other newt populations in the region may be in need of similar intervention to prevent or reduce vehicle strikes. Indeed,

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[1] https://www.ebparks.org/about-us/whats-new/news/tildens-south-park-drive-closed-annual-newt-migration

[2] https://www.pressdemocrat.com/article/news/newt-brigade-shuttles-salamanders-to-safety/

during surveys conducted over four breeding seasons spanning 2017 to 2021, a community scientist (Parsons, 2021) documented over 15,000 vehicle-killed newts, including both Bay Area *Taricha* species, along Alma Bridge Road, a small two-lane road in western Santa Clara County. These results have generated media interest and prompted investigation of potential effects of traffic mortality on the local newt populations.[3] This case is particularly intriguing because it appears that large numbers of newts have been killed on the road for many years, yet neither species have become extirpated from the area, at least not yet. Thus, it is unclear whether the newt populations can sustain these levels of road mortality and persist long-term or whether they are on a downward trajectory towards extirpation. Indeed, considerable lag time might elapse between the onset of population decline and ultimate population extirpation.

Alma Bridge Road is a 7.4-kilometer (4.6 mile) long, two-lane road along the east side of Lexington Reservoir (Figure 1). The northern section of the road is used by trucks to transport sandstone from the Lexington Quarry (Vulcan Materials Company) to the east, recreationalists (hikers, boaters, and bicyclist) that use the road for travel and parking to access Lexington Reservoir County Park and surrounding open space preserves, and private residents that have property to the south and east. The road is also used by commuters that choose to bypass a section of Highway 17, especially during times of high congestion. Observations of high newt mortality on Alma Bridge Road suggest that, if left unchecked, such traffic impacts could have an impact on this population (Parsons, 2021).

The purpose of our study is to estimate the effect of current levels of vehicular strikes on the local population of California newts, the newt species that predominates in the study area. Knowledge of these population-level effects will allow better understanding of the conservation value of any mitigation of this impact. Thus, we conducted a drift fence/pitfall trap array study to estimate the number of adult California newts attempting to cross Alma Bridge Road to breed in Lexington Reservoir and estimate the percentage that were killed by vehicular strikes during a single breeding season (2020/2021). We then incorporated these estimates into a population viability model to simulate the long-term impacts of road mortality on the local California newt population.

Materials and methods

Study area

The study area encompassed the approximately 6.6-kilometer (4.1-mile) section of Alma Bridge Road from the Saint Joseph Hill Open Space Preserve (OSP) trail (37.200364°, −121.987036°) to the junction of Aldercroft Heights Road (37.168124°, −121.980288°) (Figure 1). This section of road mostly separated the eastern shore of Lexington Reservoir from the adjacent expanse of upland habitat that is a mixture of public open space and private property. Based on the high dead-on-road (DOR) counts (Parsons, 2021), we suspected that the newt mortality on Alma Bridge Road was a result of adult newts crossing the road from upland habitat to aquatic breeding habitat during annual breeding migrations. California newts breed in ponds, streams, and reservoirs (Kuchta, 2005; Thomson et al., 2016), including Lexington Reservoir and at least one of its inlet streams, downstream from Alma Bridge Road (section 1 of Supplementary material). The area between the reservoir and the road contains little upland habitat and appears to lack other potential breeding sites. Therefore, for the purposes of our study, we defined the local population of California newts subjected to mortality on Alma Bridge Road to consist of those that inhabit upland habitat east of Lexington Reservoir, breed exclusively in Lexington Reservoir or in its inlet streams between Alma Bridge Road and the reservoir, and must cross Alma Bridge Road in order to breed. We acknowledge that some newts breeding in the reservoir may use upland habitat to the southwest (e.g., south of the reservoir or between the reservoir and Highway 17). It is also our understanding that the extension of Lexington Reservoir in Lyndon Canyon west of Highway 17 is not freely connected to the reservoir proper under Highway 17 but instead water between this extension and the reservoir must pass through a high-water Lexington Culvert under the highway north of Black Road that would restrict adult newts and preclude larval newts from freely crossing under Highway 17 between the extension and the reservoir. Therefore, due to residential development southwest of the reservoir and the presence of Highway 17 (a barrier to newt dispersal) to the west, it is our opinion that population dynamics in Lexington Reservoir as a whole are driven primarily by the newts that were explicitly included in our study. Therefore, our references to “population” in this paper refer interchangeably to the group of newts that were explicitly included in our study and the overall population breeding in the reservoir.

Drift fence/pit-fall trap survey

In late October and early November 2020, we installed six drift fence/pitfall trap arrays at the locations indicated in Figure 1. These six arrays were distributed across four spatial blocks (1–2 arrays per block). These blocks were delineated to distinguish potential differences in road use by the public and thus possible differences in traffic volume. Blocks are separated by three destinations along Alma Bridge Road. Block 1 extends from the Saint Joseph Hill OSP trail to the Los Gatos Rowing Club and includes the road segments at Arrays I and II; Block 2 extends from the Los Gatos Rowing Club to the entrance of the Miller Point Parking Lot and includes the road segment at Array III; Block 3 extends from the Miller Point Parking Lot to the Soda Springs Road junction and includes the road segments at Arrays

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[3] https://www.openspace.org/visit-a-preserve/plants-wildlife/newts

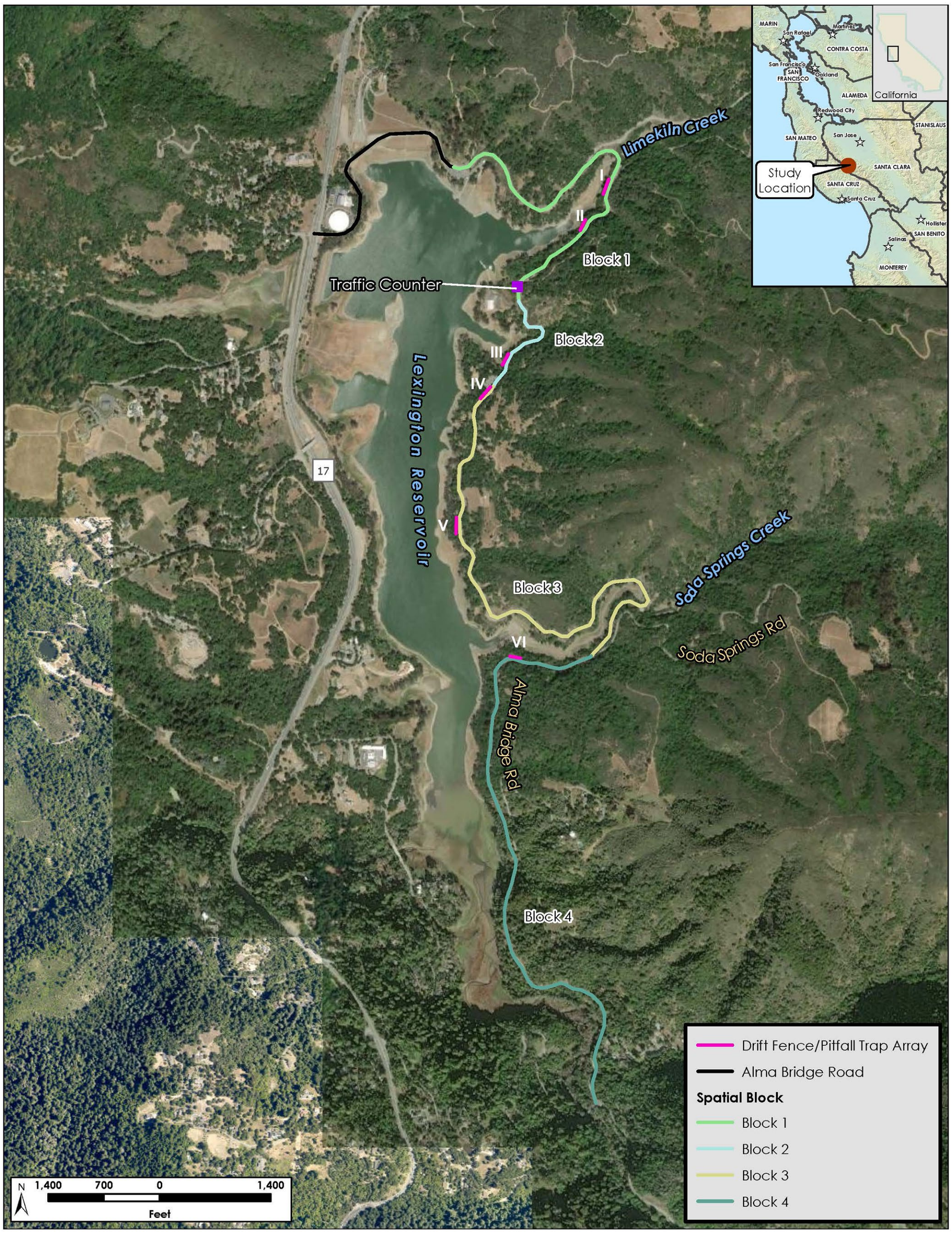

FIGURE 1
Alma Bridge Road study area, showing Lexington Reservoir, upland habitat, the road, and trapping arrays and spatial blocks along the road.

IV and V; and Block 4 extends from the Soda Springs Road junction to the intersection of Alma Bridge Road and Aldercroft Heights Road and includes the road segment at Array VI (Figure 1). Mortality rates are expected to differ between the four blocks due to differences in various aspects of vehicular traffic including the number of vehicles and the timing of travel with respect to newt activity. For example, we expect that most of the traffic to and from the Los Gatos Rowing Club comes from Highway 17, which could cause differences in traffic patterns between Block 1 and the other blocks. Although we did not measure differences in traffic volume between blocks (because we utilized only one traffic counter, see below), it is likely that they are largely responsible for the between-block differences we detected in newt mortality rates (see section Results), although other factors might also contribute to the between-block differences.

We placed each array at the downslope (reservoir) side of Alma Bridge Road to intercept and capture newts that moved from the uplands, and that successfully crossed the road, towards the reservoir. The barrier consisted of silt fence buried at least

FIGURE 2
(A) Array V showing pairs of traps on the front (upslope) and back (downslope) side of the array. **(B)** Pitfall trap at Array V flush with ground and against the array.

15.2 cm (6 inches) below ground and extending 61 cm (24 inches) above ground (Figure 2). All arrays were initially planned to be of equal length, but due to site-specific limitations (e.g., the lack of a sufficiently wide shoulder in which to construct the arrays), the arrays ranged in length from approximately 36.6 to 57.9 m (120 to 190 feet) as follows: Array I at 39.6 m (130 feet), Array II at 57.9 meters (190 feet), Array III at 57.9 meters (190 feet), Array IV at 51.8 m (170 feet), Array V at 36.6 m (120 feet), and Array VI at 36.6 m (120 feet). We installed a series of paired pitfall traps at each array; at each pair, one trap was on the upslope side (front trap) and the other trap was on the downslope side (back trap) of the array. The outermost pairs of pitfall traps were located 1.5 m from the ends of each array, with intervening pairs of traps spaced at 3-m intervals. There were a total of 24 traps (12 pairs) at Array I, 36 traps (18 pairs) at Arrays II and III, 32 traps (16 pairs) at Array IV, and 22 traps (11 pairs) at Arrays V and VI. This placement was designed to intercept both newts traveling to the reservoir to breed and newts traveling from the reservoir back to the uplands upslope of the road after breeding. The pitfall traps were flush with the array so that a newt would fall into the trap as it was walking along the array in an attempt to cross to the other side (Figure 2).

We began the surveys on 4 November 2020, conducting daily inspections of the arrays and adjacent road segments at the arrays, and ending the surveys on 31 March 2021. California and rough-skinned newts breed during the wet season, moving from upland refugia to breeding waterbodies after the fall/winter rains begin and breeding before moving back into upland areas (Stebbins and McGinnis, 2012). Therefore, we expected that the period of early November through March would encompass the breeding season for the newts and would allow us to determine the peak(s) of newt movement across the road. During each daily inspection, we inspected each road segment adjacent to an array for newt carcasses, and the road segment 15.2 m (50 ft) to the north and 15.2 m to the south of the array (north and south "wings"). As a newt carcass was identified on the road, it was photographed, georeferenced, and then removed from the road so that it was not counted subsequently. We did not attempt to identify dead newts to species, sex, or life stage, because the poor condition of most DOR individuals made determination of these variables difficult or impossible. Thus, DOR counts at each array likely include some individuals of each of the two newt species present in the area. We conducted the inspections at the same time each morning to standardize results (with a starting time of 9 a.m. at Array I). This design followed the recommendations of Santos et al. (2011), who advise that road mortality surveys for salamanders be conducted daily to minimize the negative bias in counts that arises if carcass persistence times are short. At each array, after inspecting the adjacent road segment and its "wings," we then inspected the pitfall traps for newts. We counted and categorized each newt (i.e., species, age-class, and sex) in each pitfall trap on the upslope (road) side of the array (front trap) and released the newt to the nearest suitable habitat on the downslope (reservoir) side of the array, under the assumption that newts captured in the front (road side) traps were moving toward the reservoir when they encountered the array. We then repeated this procedure for newts in the pitfall traps on the downslope (reservoir) side of the array (back trap), releasing the newts to the nearest suitable habitat on the upslope side (across the road) under the assumption that these newts were moving away from the reservoir.

Strategies to reduce wildlife road mortality have become a component of many conservation efforts (Glista et al., 2009; Beebee, 2013). However, their success depends on knowledge of the spatiotemporal patterns of mortality. Therefore, we also investigated hourly variation in traffic level and the relationship between newt road crossing attempts and weather to better understand where the 'hotspots" (i.e., areas of high mortality) and when the "hot moments" (i.e., times of high newt movement) occur to help inform the timing and location of mitigation efforts. To provide information on vehicular activity within the study area, we installed a TRAFx vehicle counter on a guard rail post at 37.196278° -121.984222° between Arrays II and III (Figure 1). The counter was able to take hourly counts of vehicles in both directions over the survey period. We tabulated the hourly counts into daily totals corresponding to the periods in which newt observations were tallied (i.e., the 24 h period from 9:00 a.m. of the previous day to 9:00 a.m. of the day of each newt survey). We also took daily rainfall data from the National Ocean and Atmospheric Administration Lake Kittridge, CA KDGC1 station, approximately 2.6 km west of the study area. The period of recorded precipitation associated with each particular date of newt observations was from 9:15 a.m. of the previous day to 9:00 a.m. of the date of newt survey.

Data analysis

Determining mortality rates and numbers of adult California newts attempting to cross Alma bridge road at arrays

The main analytical objectives of this study were to measure the road-induced mortality rate of California newts attempting to cross Alma Bridge Road and determine whether this mortality is likely to adversely affect the Lexington Reservoir California newt population. Accordingly, we (1) measured the mortality rates of newts at multiple road segments (the arrays), (2) investigated a set of statistical models that related mortality rate to potential explanatory variables, (3) selected from this set a well-supported model that related mortality rate to road location (block), and (4) applied array results and the model to estimate the total number of adult California newts for the whole road (population size) and derive overall road mortality results for the population. We arrived at a single ratio of adult newts killed on the road to the total number of adult newts attempting to cross the road during the breeding migration. We also estimated the ratio of juvenile newts killed on the road to the total number of juveniles attempting to disperse across the road to access upland habitat following metamorphosis. We then incorporated these ratios and our estimate of population size into the population viability model of Gibbs and Shriver (2005) to test the influence of road mortality.

Fortunately for us, annual DOR carcass surveys, started in 2017, were continued by community scientists during the 2020/2021 breeding season concurrently with our pitfall trap surveys.[4] Therefore, we were able to combine the 2020/2021 DOR data with our 2020/2021 pitfall trap results to estimate an adult newt breeding number and overall road-based mortality rate of the newts crossing Alma Bridge Road to breed in Lexington Reservoir. The two studies were closely coordinated to avoid duplication of efforts, to avoid double counting of carcasses, and to better inform both studies. Based on the pitfall trap captures (see section Results), which indicated that the vast majority of newts crossing Alma Bridge Road were California newts, we decided to restrict further analysis to this species.

To facilitate data analysis, we made a series of assumptions based on our understanding of California newt breeding biology, breeding migration, array position, survey methodology, and model requirements (Assumptions S1-S7, section 2 in Supplementary material). With these assumptions, we calculated the number of adult California newts attempting to cross the road ($A_{at,ur}$) at each array to breed in the reservoir with the following equation:

$$A_{at,ur} = T_{at,ur} + D_{ur} \times C_{at,ur} \tag{1}$$

where $T_{at,ur}$ is the number of adult California newts captured in the front traps (traps on the upland side of the array), D_{ur} is the number of newts DOR at the array, and $C_{at,ur}$ is the proportion all newts (including both species, both sexes, and all life stages) captured in the front traps that were adult California newts. This equation applies Assumption S4 (section 2, Supplementary material).

$C_{at,ur}$ is calculated separately for each array using the following equation:

$$C_{at,ur} = T_{at,ur} \,/\, \left(T_{at,ur} + T_{jt,ur} + T_{ag,ur} + T_{jg,ur}\right) \tag{2}$$

where $T_{jt,ur}$ is the number of juvenile California newts, $T_{ag,ur}$ is the number of adult rough-skinned newts, and $T_{jg,ur}$ is the number of juvenile rough-skinned newts captured in the front traps. We then calculated the survival rate of breeding adult California newts crossing the road at each array to breed in the reservoir ($S_{at,ur}$) with:

$$S_{at,ur} = T_{at,ur} \,/\, A_{at,ur} \tag{3}$$

The corresponding mortality rate ($M_{at,ur}$) was calculated thus:

$$M_{at,ur} = 1 - S_{at,ur} \tag{4}$$

The number of adult California newts that died attempting to cross the road in the upland-to-reservoir direction ($D_{at,ur}$) was estimated as:

$$D_{at,ur} = M_{at,ur} \times A_{at,ur} \tag{5}$$

We were able to directly calculate the number of adult California newts attempting to cross the road in the reservoir-to-upland direction after breeding in the reservoir ($A_{at,ru}$) at each array by totaling the number of adult California newts captured in the back traps (traps on the reservoir side of the array) over the entire survey period. However, because the arrays prevented newts from crossing the road at the arrays, and surveyors transported newts captured in the back traps across the road, we used an

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[4] https://www.inaturalist.org/projects/pacific-newt-roadkill-main-project-lexington-reservoir

indirect method to calculate the mortality rate of adult California newts crossing the road after breeding in the reservoir as follows.

We first divided the survey period into 144 one-day periods; as described above, this was done to control for potential differences in mortality rate between directions arising from daily variation in traffic levels and differential timing and location of newt movement between the two directions. Although the survey period lasted 148 days (21 weeks and 1 day), we excluded the first 4 days because traffic counts necessary for the regression modeling of newt mortality did not begin until the fifth day, and no newts were observed in the traps or on the road at the arrays during the initial four-day period. Each one-day period began after the morning check and lasted to the end of the morning check on the following day.

For each combination of day and array, we obtained a prediction of the mortality rate of all newts (all species, life stages, and sexes) attempting to cross the road in the upland-to-reservoir direction on that particular day at that particular array ($M_{\boldsymbol{d},ur}$) using an array-based logistical regression model (array model, sections 3 and 4 of Supplementary material).

We then applied Assumption S6 (section 2 of Supplementary material), wherein we assumed that the mortality rate for adult California newts attempting to cross the road at an array in the reservoir-to-upland direction on a particular day ($M_{\boldsymbol{d},\boldsymbol{ru}}$) would be equal to $M_{\boldsymbol{d},ur}$ (the mortality rate predicted by the array model for that particular array on that particular day). Next, for each day/array combination, we multiplied the number of adult California newts captured in the back traps by the appropriate $M_{\boldsymbol{d},\boldsymbol{ru}}$ value to estimate how many would have been killed on that part of the road that day if the array was not present (i.e., "rescued" adult California newts). For day/array combinations in which no adult California newts were captured in the back traps, the number of rescued adult California newts was zero.

For each array, we summed the number of "rescued" adult California newts across all 144 day periods to derive the total number of "rescued" adult California newts ($R_{at,\boldsymbol{ru}}$). We then calculated the estimated mortality rate for the array over the study period for the reservoir-to-upland direction ($M_{at,ru}$) by dividing $R_{at,\boldsymbol{ru}}$ by the total number adult California newts attempting to cross the road in that direction (total number of adult California newts captured in the back traps) ($A_{at,ru}$).

Determining mortality rates and numbers of adult California newts attempting to cross Alma Bridge Road in the entire study area

We used the array results, combined with the community scientist data from the same season (2020/2021), to estimate the number of adult California newts attempting to cross Alma Bridge Road in the entire study area when migrating to Lexington Reservoir to breed and the number killed during this breeding migration to, and the reverse migration from, Lexington Reservoir due to vehicular strikes. Because the arrays represent only a portion of Alma Bridge Road, we used statistical models to (1) investigate factors potentially associated with mortality of California newts attempting to cross the road at the arrays, and (2) select the best approach for applying the array-based mortality rates to the other parts of the road.

Statistical models of road mortality and calculation of whole-road mortality rates

We estimated overall, whole-road mortality rates for adult California newts attempting to cross Alma Bridge Road in each direction and the number of adult California newts attempting to breed by combining array results with the results of community scientist observations of DOR newts in road areas outside of the arrays. Rather than assume that mortality rates were constant in time and space, we used statistical models to derive the relationship between mortality and environmental variables. These models, which indicated that mortality rates were not constant, helped us derive rigorous estimates of the whole-road mortality rates and the number of California newts attempting to breed.

In brief, we used regression to analyze the outcome of individual road-crossing attempts at arrays to investigate block and other environmental variables (day, daily number of vehicles, percent cover of uncanopied grassland, canopy cover, average slope, and average aspect) as potentially explanatory variables (sections 3 and 4 of Supplementary material). Based on Aikaike information criterion (AIC) scores, the "complex" model, which contained block, day, and daily number of vehicles as explanatory variables, was the best model for describing mortality of newts attempting to cross the road in the upland-to-reservoir direction (Table 1; section 4 of Supplementary material). This model was selected as the "array" model and was used to estimate mortality rates for adult newts traveling in the opposite (reservoir-to-upland) direction (see Assumption S6, section 2 of Supplementary material) and to build the binomial dataset for that direction (See section 3 of Supplementary material). The "non-block" model, which contained day, daily number of vehicles, and canopy cover as explanatory variables, was the best model for describing mortality of newts attempting to cross the road in the reservoir-to-upland direction (Supplementary Table S1).

Ideally, we would apply the complex and non-block models to the community scientist observations to estimate mortality rates for the non-array road areas. However, this approach was not possible because both models included day and number of vehicles (a daily count) as explanatory variables. These variables are absent from the community scientist dataset and cannot be incorporated into it, because the community scientist observations were not made every day. Thus, we applied the "block-only" models (sections 3 and 4 of Supplementary material), which outperformed the non-block models and did not require day or daily number of vehicles as model inputs.

We applied the block-only models to obtain direction-specific estimates of the number of California newts attempting to cross the road and the number that died in the attempt for areas located outside of the arrays, using (1), the array-derived mortality rates that were specific to block as well as the newts' direction of travel,

TABLE 1 Number of newts captured and observed DOR at arrays.

	Front (Road Side) Traps						Back (Reservoir Side) Traps								
	TT			TG			TT			TG			DOR		
Array	M	F	J	M	F	J	M	F	J	M	F	J	Array	W1	W2
I	160	83	18	6	2	0	38	75	10	1	3	1	92	61	84
II	121	126	25	5	2	0	75	171	22	0	2	2	92	38	31
III	209	164	34	3	9	0	94	173	53	0	0	0	91	27	26
IV	85	45	13	1	0	0	28	37	8	0	0	0	47	16	23
V	18	30	0	0	0	0	14	30	3	0	0	0	12	11	5
VI	81	82	5	1	2	3	33	82	11	1	0	2	42	36	44
Total	674	530	95	16	15	3	282	568	107	2	5	5	376	189	213

TT, *Taricha torosa* (California newt); TG, *Taricha granulosa* (rough-skinned newt); M, male; F, female; J, juvenile; DOR, dead on road, W1, north wing; W2, South wing at each array.

and (2) community scientist counts of DOR newts outside of the arrays (section 5 of Supplementary material). Then, for each direction of newt travel, we totaled the array and non-array estimates of dead and "rescued" adult California newts across all four blocks to obtain an entire-road estimate of overall adult California newts that died on the road. Thus, "rescued" adult California newts were counted as dead, to best estimate what the overall mortality would have been without intervention. We also, for each direction of newt travel, totaled the array and non-array results for number of adult California newts attempting to cross the road across all four blocks. We then calculated an overall mortality rate and an overall survival rate (1 – overall mortality rate) for each direction of newt travel from the entire-road estimates of the number of adult California newts that died on the road and the number that attempted to cross the road. The overall mortality rate for adults attempting to cross the road to breed during the 2020/2021 breeding season was calculated by subtracting the product of the survival rates in each direction from one.

We then applied the estimated number of adult California newts attempting to cross Alma Bridge Road in the upland-to-reservoir direction in order to breed in Lexington Reservoir and the overall mortality rate as two parameters from this study to be used in the aforementioned population viability model under the following assumption:

1. The local population of California newts that reside in the upland habitat at Lexington Reservoir consists of individuals that exclusively breed in Lexington Reservoir and are required to cross Alma Bridge Road in order to breed.

Projecting the impact of road-based mortality on the Lexington reservoir California newt population using a population viability model

We used a model of population viability in order to investigate the impact of this road-related mortality on the local newt population (Gibbs and Shriver, 2005). We identified the parameters and input data that were required to construct this model of population viability to determine the threshold level at which such mortality would be great enough to risk long-term population viability specific for the population at Alma Bridge Road. Some of the data were available from the literature, but other data would need to be obtained through additional fieldwork or by making assumptions.

The model from Gibbs and Shriver (2005) is:

$$N_{a,t}+1 = N_{a,t} \times \sigma_a + \sigma_m \left(K_l - N_{e,t}\right) K_l \times \sigma_j \tag{6}$$

where $N_{a,t}$ is the number of adults in the current year, $N_{e,t}$ is the number of eggs produced in a given year, σ_a is the adult annual survival rate, σ_m is the survival rate from egg to metamorphosis, σ_j is the survival rate of juveniles through their first winter, and K_l is the carrying capacity of larval habitat.

The number of eggs produced in a given year is calculated as:

$$N_{e,t+1} = N_{e,t} \times \sigma_a \, \phi_m \tag{7}$$

where ϕ_m is the average eggs produced per individual, calculated by multiplying the number of eggs per mass by the number of masses laid per year, and multiplying this number by the probability of successful breeding by females after arrival at the breeding site divided by 2 (to account for the presence of both sexes).

The starting adult population (or current population during a given year) was provided in this study. From this starting adult population, a starting average number of eggs ϕ_m was calculated by multiplying the number of individuals with the average clutch size and the average number of clutches per year. For the California newt, this average number of eggs was not available in the literature; instead the literature provided a range of 7–47 eggs per egg mass and a range of 3–6 egg masses laid by a female during the breeding season (Brame Jr., 1968) in addition to an ovarian count range of 130–160 per female (Thomson et al., 2016). We therefore made the assumption that the average number of egg masses laid is 4.5, with an average number of eggs per egg mass of 32.2, to arrive at a midrange ovarian count of 145.

A study by Jones et al. (2017) used a much lower average of 60 eggs (24 eggs in 2.5 egg masses) laid per female during a breeding season. They base this average 60 eggs laid per female on Kats et al. (2013) who state in the introduction that "after breeding, female newts remain in the streams to deposit small (2–3 cm diameter, 15–30 embryos each) egg masses…"; and on Brame Jr (1968) who referenced Ritter (1897) stating that the average number of eggs produced at any one laying… "seems to be about 60 for each female, these being distributed in three or four of the masses." Yet, Brame in the same study observed one female laying six egg masses, with an average of 22.1 eggs per mass for a total of 133 eggs, and suggested that his higher counts may reflect differences in geographic regions between his observations and those of Ritter. We however, consider our average of 145 eggs (32.2 eggs per 4.5 egg masses) to be a better indicator of average number of eggs laid by females based on an egg mass range of 3–6 egg masses and an ovarian count range of 130–160 per female as provided in Thomson et al. (2016). Therefore, we kept our average number of 145 eggs per female instead of the much lower 60 eggs per female.

Jones et al. (2017) suggested that females migrate to breeding sites annually and breed through most of their adult lives but that the probability of successful breeding depends on the availability of appropriate breeding habitat. However, Thomson et al. (2016) state that it is unknown whether females breed every year or skip years, and Stebbins and McGinnis (2012) state that "individual newts probably do not breed every year." Similarly, they state that for red-bellied newts "males breed every year, but females rarely breed more frequently than at two- or three-year intervals or longer." This statement is based on mark-recapture research on homing ability in red-bellied newts, with an interpretation of results speculating on the frequency of breeding by males and females (Twitty, 1961, 1964). Therefore, to be conservative, we decided to set the probability of successful breeding by females, given that they survive the breeding migration and arrive at the breeding site, to 0.5. However, we assumed that females and males in our study population migrate to breeding sites annually because the sex ratio we observed in migrating adults was approximately equal (see section Results).

Also, because we were unable to find a previously reported survival rate from egg to metamorphosis in the literature, we followed Jones et al. (2017) in using a proxy of 0.025 for tiger salamanders (*Ambystoma tigrinum*). This rate may correspond to the potential effects of predation by fish in the reservoir. Crayfish and mosquitofish (*Gambusia affinis*) are known to prey on newt larvae (Gamradt and Kats, 1996), which do not retain the toxin present in the egg mass after yolk absorption (Twitty and Johnson, 1934; Twitty, 1937). Other predatory fishes, such as native rainbow trout (*Oncorhynchus mykiss*) and nonnative green sunfish (*Lepomis cyanellus*), are commonly abundant in streams devoid of newts, indicating that these fish may exclude newts in some situations (Kuchta, 2005). These fishes are present in Lexington Reservoir[5], suggesting that predation might be a significant source of larval mortality in our study population.

Carrying capacity of the reservoir for California newts is unknown. Due to the size of the reservoir, one could assume that the carrying capacity is very large (e.g., into the millions of larvae). However, carrying capacity depends on the abundance of aquatic invertebrate food source for the larvae in the reservoir, which may be quite small, as reservoirs are considered less productive than other natural waterbodies of the same size typically due to a limited littoral zone (Moss, 2008). We therefore, provided a carrying capacity of the reservoir as the larval habitat of 2,000,000 (an admittedly speculative number estimated as four times the starting number of eggs).

To model the population in the absence of road mortality, we used a baseline value of σ_j (survival rate of juveniles through their first winter) equal to 0.7917, which was taken from the results of Jones et al. (2017). Following Jones et al. (2017), baseline σ_a (adult annual survival) was set at 0.91, a value based on a study on red-bellied newts by Twitty (1966). To model the population in the presence of mortality on Alma Bridge Road, we recalculated σ_j and σ_a under the following assumptions:

2. A juvenile newt will cross the road exactly once when dispersing to the upland from the reservoir after metamorphosis.
3. Adult newts attempting to breed attempt to cross the road once in the upland to reservoir direction. The adults that attempt to cross the road in the upland to reservoir direction during a breeding migration subsequently attempt to cross the road in the other direction to return to the upland habitat.
4. Each attempted road crossing is a separate event unrelated to the baseline mortality rate.

Thus, for the road mortality scenario, σ_j was calculated as the baseline σ_j multiplied by the overall survival rate of newts crossing in the reservoir to upland direction, and σ_a was set equal to baseline σ_a multiplied by the overall survival rate of newts crossing in the reservoir to upland direction and the overall rate of survival rate of newts crossing in the other direction.

Results

We captured a total of 2,302 newts in the pit fall traps: 1,333 newts in the upslope or road side (front) traps, and 969 newts in the downslope or reservoir side (back) traps. We also counted 376 newts DOR at the arrays and an additional 402 newts DOR at the wings. Of the newts captured in the traps, 2,256 (98%) were California newts and 45 (2%) were rough-skinned newts. Of the California newts captured, 956 (42%) were adult males, 1,098 (49%) were adult females, and 202 (9%) were juveniles (individuals that were under 2.5 inches total length and lacked breeding adult characteristics such as smooth skin and flattened tail in both sexes, and enlarged tailfin, swollen cloaca, and nuptial pads on finger tips in males). Of the

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[5] https://parks.sccgov.org/plan-your-visit/activities/fishing

TABLE 2 Standardized numbers of newts captured and observed DOR at arrays.

	Front (Road Side) Traps						Back (Reservoir Side) Traps								
	TT			TG			TT			TG			DOR		
Array	M	F	J	M	F	J	M	F	J	M	F	J	Array	W1	W2
I	234	121	26	9	3	0	56	110	15	1	4	1	134	89	123
II	121	126	25	5	2	0	75	171	22	0	2	2	92	38	31
III	209	164	34	3	9	0	94	173	53	0	0	0	91	27	26
IV	95	50	15	1	0	0	31	41	9	0	0	0	53	18	26
V	29	48	0	0	0	0	22	48	5	0	0	0	19	17	8
VI	128	130	8	2	3	5	52	130	17	2	0	3	67	57	70
Total	816	639	108	19	17	5	330	672	121	3	6	6	455	246	283

TT, *Taricha torosa* (California newt); TG, *Taricha granulosa* (rough-skinned newt); M, male; F, female; J, juvenile; DOR, dead on road, W1, north wing; W2, South wing at each array.

TABLE 3 Number of adult California newts caught in front (road side) traps, contribution to all newts caught in front (road side) traps, estimated number attempting to cross Alma Bridge Road in upland to reservoir direction at each array, and survival and mortality rates for that direction.

Array	Number in front (road side) traps	Contribution	Total number	DOR	Survival rate	Mortality rate
I	243	0.90	326	83	0.75	0.25
II	247	0.89	328	81	0.75	0.25
III	373	0.89	454	81	0.82	0.18
IV	130	0.90	172	42	0.75	0.25
V	48	1.00	60	12	0.80	0.20
VI	163	0.94	202	39	0.81	0.19

rough-skinned newts captured, 18 (40%) were adult males, 20 (44%) were adult females, and 8 (16%) were juveniles. A breakdown of the numbers of newts observed DOR and captured at each array is provided in Table 1. Here, because the arrays differed in length, we standardized results for each array by multiplying the number of individuals capture at that array and DOR at that array by 57.9 (the length of the longest array in meters) divided by the length of the target array. These standardized numbers are provided in Table 2. These standardized numbers indicate that Array I has the highest DOR counts, followed by Arrays II and III, then Array VI, Array IV, and finally Array V. At least 17 other vertebrate species were observed during array-based and community scientist surveys during the 2020/2021 newt breeding season, but the carcasses of each non-newt vertebrate species were at least two orders of magnitude less abundant than newt carcasses (section 9 of Supplementary material).

Adult California newt crossing attempts and road crossing mortality rates at arrays

Table 3 provides the estimates of the number of adult California newts attempting to cross Alma Bridge Road at the arrays in the upland-to-reservoir direction and the mortality rates due to vehicular strikes. These adult California newt numbers and mortality rates are directly calculated by the number of adult California newts captured in the front traps, contribution of adult California newts to all newts captured in the front traps, and newts DOR at each array. The mortality rates are relatively similar between the six arrays (range of 18 to 25 percent) but are higher at Arrays I, II, and IV than at Arrays III, V, and VI. The higher mortality rates at Arrays I, II, and IV, even though the total number of newts crossing the road at Array III is the highest among all arrays, indicate that the road segments at Arrays I, II, and IV were possibly experiencing higher vehicular use due to their locations at or near recreation-associated parking areas (which are not present in the other segments).

Table 4 provides the number of adult California newts attempting to cross Alma Bridge Road at the arrays in the reservoir-to-upland direction based on the number of adult California newts captured in the back (reservoir side) traps, and the estimated number of "rescued" adult California newts based on the day-specific rates of mortality from vehicular strikes for newts attempting to cross the road predicted by the array model. Estimated mortality rates for Arrays I, IV, V, and VI were very similar, falling within a range of 6 percentage points (19–25%). Estimated mortality rate was relatively high at Array II (27%) and relatively low at Array III (16%) because of two factors: (1) the directly observed mortality rates for the upland-to-reservoir direction were relatively high at Block 1 arrays (Arrays I and II)

TABLE 4 Number of adult California newts caught in back (reservoir side) traps, estimated number of "rescued adult California newts", and mortality rate at each array for adult California newts attempting to cross Alma Bridge Road in reservoir-to-upland direction.

Array	Number in back (reservoir side) Traps	Total number of rescued adult California Newts	Estimated mortality rate
I	113	29	0.25
II	246	65	0.27
III	267	44	0.16
IV	65	14	0.22
V	44	10	0.23
VI	115	22	0.19

and relatively low at the single array in Block 2 (Array III), which heavily influenced the array model upon which the reservoir-to-upland estimates were based; and (2) newts attempting to cross the road in the reservoir-to-upland direction at Arrays II and III happened to time their crossings on days that also happened to be relatively high and relatively low, respectively, in risk of road mortality. Nevertheless, mortality rates for the reservoir-to-upland direction were fairly consistent; all rates for this direction fell within a range of 11 percentage points (16 to 27%). Thus, across our estimates of mortality for each direction at each of the six arrays, there was a difference of only 11 percentage points between the highest and lowest estimate. This consistency suggests that our sampling regime was sufficient in geographic scope to represent the range of variation in mortality rate along the entire road and further suggests that our estimates of whole-road mortality rates for each direction of travel, which are derived from the array-level estimates, are reasonably accurate.

Adult California newt crossing attempts and overall mortality rate

Based on our analyses, which use the newt capture results from the arrays in combination with observations of newts DOR at the arrays and segments of Alma Bridge Road located outside of the arrays, we estimate that at least 13,786 adult California newts attempted to cross the entire Alma Bridge Road in the study area in order to migrate to Lexington Reservoir to breed during the survey period. This number is the sum of our estimates of the number of adult California newts that attempted to cross the road at each array and the number that attempted to cross at the road segments of the four different blocks that are outside of the arrays. Applying the mortality rates estimated for each array and each non-array road segment, we also estimate that 3,066 adult California newts died on the road due to vehicular strikes during this migration, for a road-based mortality rate of 22.2%. Also, based on our captures of newts moving in the reservoir-to-upland direction, in combination with DOR observations from the non-array road segments, we estimate road-based mortality rate for this return migration to be 21.9%. These estimates indicate that of the 13,786 adult California newts migrating from the upland across Alma Bridge Road to the reservoir to breed, 10,720 adult California newts successfully crossed and potentially bred in the reservoir. We presume that a large proportion of these adult California newts then attempted to cross Alma Bridge Road on the return migration back to the uplands, though many were likely still on the reservoir side of the road at the end of the survey period. It is likely that these adult California newts will continue to migrate back to the uplands as conditions allow during the summer and fall, for example, during rain events or periods of high humidity at night (i.e., foggy nights).

The areas of high mortality, shown in Figure 3, were at the sections of Alma Bridge Road from Limekiln Creek south to half way between Arrays II and III, then from the curve and road section north of the Miller Point parking lot, including Array III but before Array IV, and from the junction with Soda Springs Road westward along the south side of Soda Springs Creek (including Array VI), with "hotspots" of very high mortality before and after Array I (Figure 3). Because an adult breeding California newt is required to cross the road twice to successfully survive a round trip migration, it is subject to an upland-to-reservoir mortality and a reservoir-to-upland mortality. We calculated the roundtrip survival rate of crossing the road to be 60.8% percent and therefore estimate the overall road mortality rate for migrations in both directions during the survey period to be 39.2%.

Population viability model and expected impact of road mortality on the Lexington reservoir California newt population

We incorporated these values of adult California newts attempting to cross the road in the upland-to-reservoir direction (13,786) and the estimated road mortality rates into the parameters of the population viability model of Gibbs and Shriver (2005), with all other values coming from the literature as provided in the section Materials and Methods, to determine how the Lexington Reservoir California newt population is expected to change given these new model inputs.

The graph in Figure 4 illustrates the results. As indicated in this graph, the population is predicted to quickly decline to under

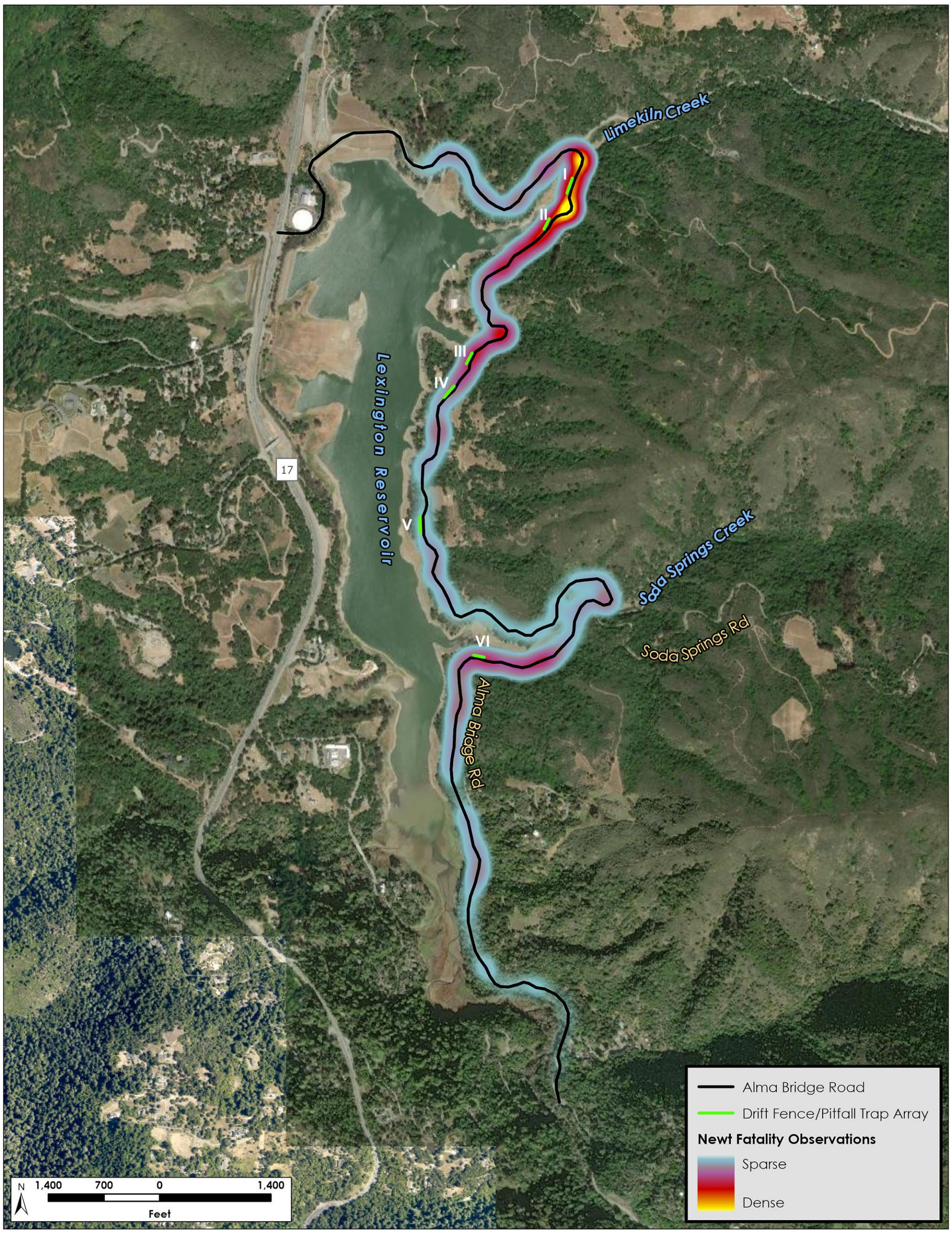

FIGURE 3
Newt mortality density map.

1,000 adult California newts in 23 years and may be extirpated in 92 years (red trend line in Figure 4).

Correlation of newt movement with precipitation

Of the 148 days of the survey period, 45 days received precipitation. These "rain days" were distributed as 11 single days, four pairs of consecutive rain days, three groups of three, three groups of four, and a single group of five consecutive rain days, interspersed with days of no measurable precipitation "dry days." The longest period of consecutive dry days was a 22-day period from 20 November 2020 to 11 December 2020, followed by two 13-day periods from 9 January 2021 to 21 January 21 2021 and from 21 February 2021 to 4 March 2021, and a 10-day period from 22 March 2021 to the end of the survey period of 31 March 2021.

As expected, the majority of newt capture and newt DOR observations at arrays occurred during and after rain days, and the counts of newt capture and/or DOR observations decreased

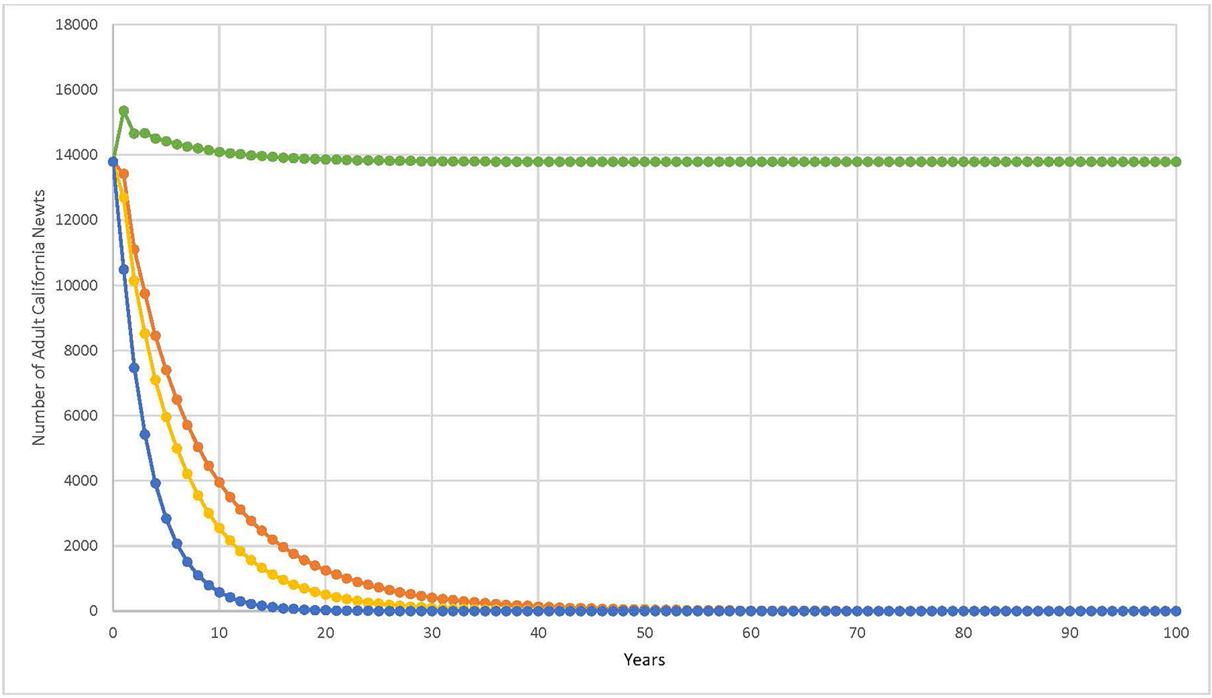

FIGURE 4
Population viability model with starting adult California newt number and mortality rate from current study. Red trend line using parameters in current study. Green trend line using parameters in current study, but changing to breeding every year. Yellow trend line using parameters in current study, but changing breeding every year and average of 60 eggs per female. Blue trend line using parameters in current study, but changing average of 60 eggs per female.

(with a few exceptions) as the period of consecutive dry days lengthened. The longest string of consecutive days with counts of newt captures and DOR is 34 days from 22 January to 24 February 2021, with a total count of 1,287 newts. This period also contained 18 rain days, with two of four consecutive rain days (27 and 28 January) receiving 2.96 and 4.21 inches of rain, respectively, the highest single-day rain totals in the survey period. This period also contained the only five consecutive rain day period from 12 February to 16 February 2021.

The highest single-day count of newts captured and DOR was 6 March 2021 at 278. This date was near the beginning of a 26-day string of consecutive days with counts of newt captures and DOR, totaling 836. This period also contained nine rain days (three single rain days, one pair of consecutive rain days, and one group of three consecutive rain days) interspersed with 18 dry days. In fact, 59 newts were captured and observed DOR in the 10 consecutive dry day period that extended to the end of the survey period (Figure 5), indicating that newts continued to move until the end of the survey period even though major rain events were over for the season.

Approximately half of the newts were observed crossing the road at the arrays (i.e., observed captured in traps and/or DOR at arrays and wings) during just 12 of the 148 days (8%) of the survey period, all associated with rain events (only one of these 12 days was immediately before a rain event). If we were to include all rain events plus two dry days after the last rain day in order to include a lag time of newt movement after a rain event, this would encompass 81 days (or 55% of the survey period) and, 91% of newt movement across the road at the arrays. So, approximately 50% of newt movement occurred during 8% of the survey period, and 91% occurred during 55% of the survey period, associated with rain events.

There also appeared to be a switch in the relative abundance of newt movement from one direction to the other at the beginning of the survey period and near the end of the survey period during the last week in February. During the initial rain events from November 2020 to 12 December 2020 more newts were captured in the back (reservoir side) traps than DOR at the arrays and/or captured in the front (road side) traps (Figure 5). Then from 13 December 2020 to 20 February 2021, more newts were typically recorded DOR at the arrays and/or captured in the front (road side) traps than captured in the back (reservoir side) traps (Figure 5). Then, from 21 February 2021 to the end of the survey period on 31 March 2021 more newts were typically captured in the back (reservoir side) traps than DOR at the arrays and/or captured in the front (road side) traps (Figure 5).

We interpret these patterns as follows: (1) newts were moving in both directions and crossing the road throughout the rainy season; however, (2) from the initiation of rain events in the fall to 12 December 2020 (day 39 of the survey period), most newts crossing the road were adult newts that most likely bred in the previous year and may have been continuing to migrate

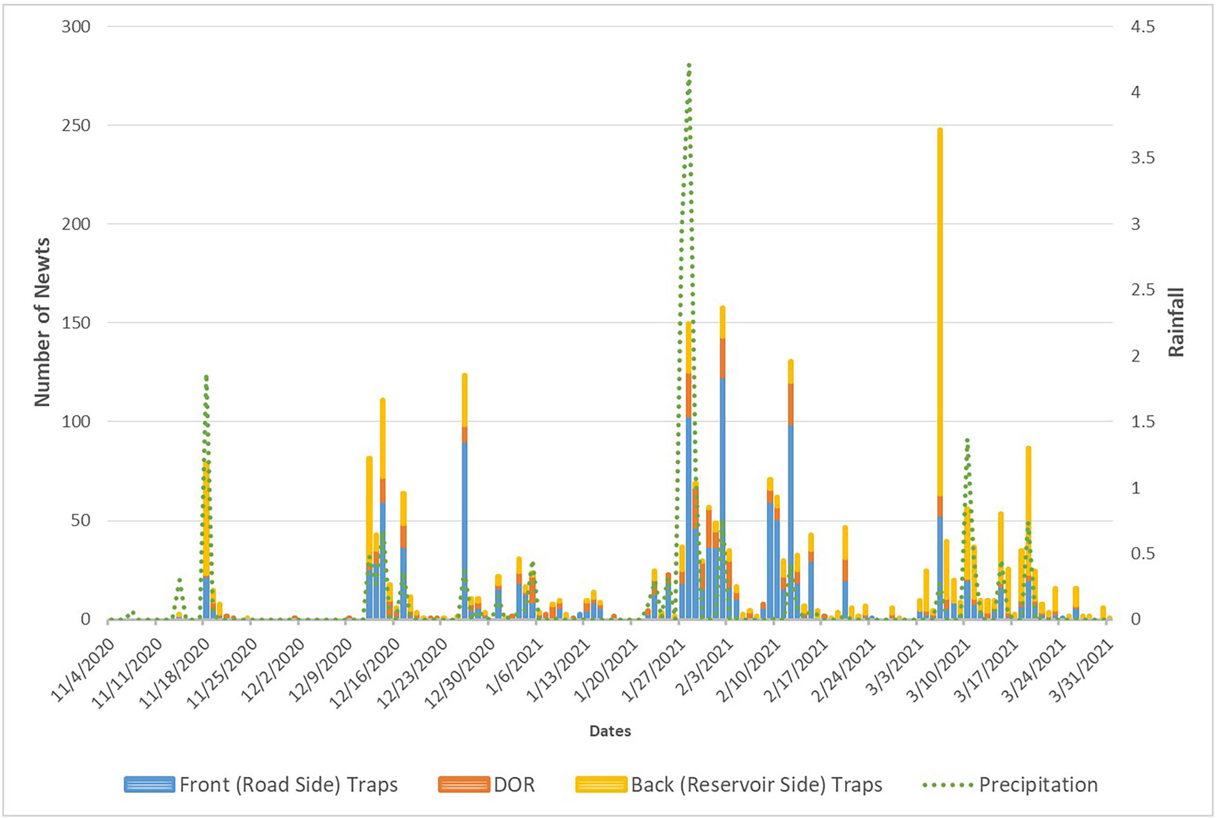

FIGURE 5
Graph of newt capture and DOR at arrays in relation with precipitation.

away from the reservoir to the upland; then, (3) from 13 December 2020 (day 40) to 20 February 2021 (day 109) most newts crossing the road were adults migrating from the upland to the reservoir to breed during this breeding season, with the peak of this migration occurring around 2 February 2021 (day 91); finally (4) from 21 February 2021 (day 110) to the end of the survey period (day 148), most newts crossing the road were adults migrating from the reservoir to the uplands after having bred during this breeding season, with a peak of this reverse migration occurring on 3 March 2021 (day 121; Figure 6). We also conclude that the rain events and 2 day lag time during early February and early March would constitute "hot moments" of newt movement.

Discussion

The results of the population viability model indicate that the local California newt population using Lexington Reservoir for breeding may be extirpated in 92 years. However, Alma Bridge Road has been in use for approximately 67 years. Therefore, it is important to discuss why this population is still extant and experiencing such a potentially high road-related mortality rate.

One reason may be that the road-based mortality rate was much lower in the past and has increased due to increased traffic volume on Alma Bridge Road. During our study there was an average of 577 vehicles per day (see section 7 of Supplementary material). This current traffic volume may have resulted from the start of and increased operations of the Lexington Quarry, increased recreational traffic with the opening of the Los Gatos Rowing Club and trails and park through the open space, and an expansion of private development in the south. The road may also have received increased overflow traffic from Highway 17 when it has become congested over the years. We have very little historical data on traffic using this road since its construction. For example, one set of data over a three-day period from 13 October through 15 October 2015 that we have examined (internal records from Santa Clara County) gives an average daily count of 194 vehicles. This count is below the lowest daily count in our study (233). Another more recent two-day count from 15 March through 16 March 2019 has an average daily count of 289 (internal records from Midpeninsula Regional Open Space District provided on 3 June 2021). If more data are available and show that traffic volume has steadily or dramatically increased over the decades then it is likely that the road-based mortality rate has also increased in concert with this increased traffic volume.

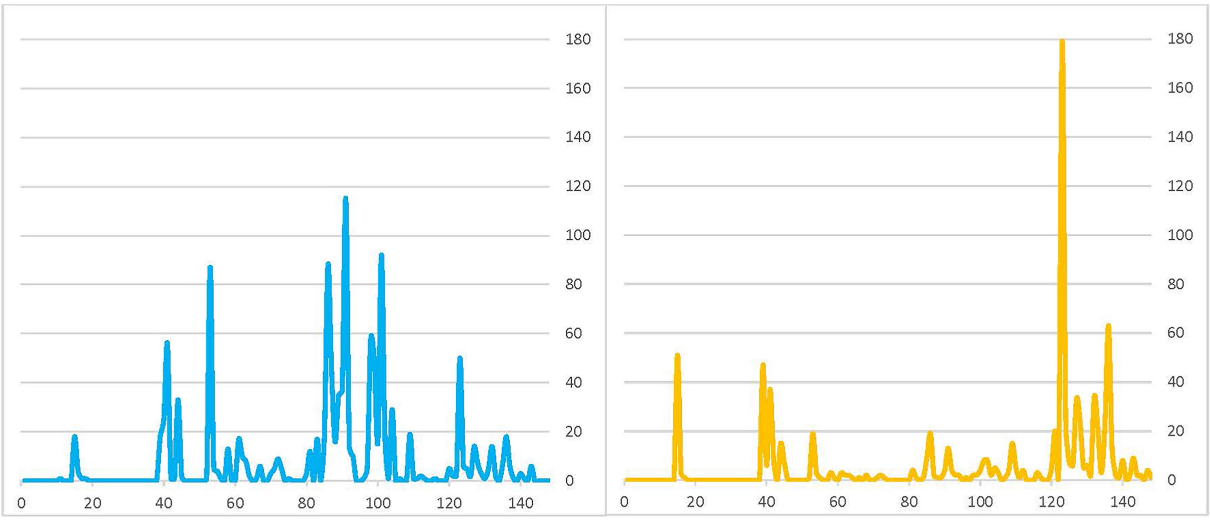

FIGURE 6
Graph of numbers of adult newts caught in front (road side) traps (blue) versus back (reservoir side) traps (orange) over the 148-day survey period.

Another possibility of why the newt population is extant is a higher annual breeding potential of the females than we used in the model. If we were to re-run the population viability model, increasing the probability of success in a female's breeding attempt, given that she reaches the breeding site, from 0.5 (as assumed in our original model) to 1.0 (all breeding attempts by females at the breeding site are successful) the adult population size would be sustained at approximately the same level in 60 years (green trend line in Figure 4). Jones et al. (2017), like our study, assumed that females attempt breeding annually. But Jones et al. (2017) also reasoned that the success of female breeding attempts is limited by the amount of habitat available for egg laying, particularly in the streams they were investigating; egg laying habitat decreases in these streams during drought conditions. We assume that in the case of Lexington Reservoir, there is not a limitation in egg-laying habitat, as the reservoir will maintain the littoral zone that newts would use regardless of annual rainfall, and therefore would be available every time a female attempts to breed and lay eggs. Therefore, it is possible that adult females achieve close to 100% success during breeding attempts after reaching the breeding site, given that Lexington Reservoir is available to them for breeding and egg laying each year, and that this breeding frequency is sufficient to sustain the population (though at a lower number) despite the high mortality rates we estimated.

However, if we were to decrease the average number of eggs per female to 60 as in the Jones et al. (2017) study, even with successful breeding occurring annually, the model results would be similar to our original results (yellow trend line in Figure 4), with the population becoming extirpated in 63 years; and if successful breeding occurred every other year, the population would be extirpated in 32 years (blue trend line in Figure 4). This exercise illustrates the importance of knowing these other parameters, particularly the average annual reproductive output of the female.

Our study assumes that all adults in the California newt population attempt to breed (i.e., undergo the breeding migration) every year. In some salamander populations, males may attempt to breed every year while females skip at least some years between attempts, foregoing the breeding migration in some years in order to avoid unfavorable conditions or to acquire energy for use in later breeding attempts. Such a scenario should result in a male-skewed sex ratio among adult salamanders undergoing the breeding migration. In contrast, we observed an approximately equal sex ratio (1.15 females, 1 male) among migrating adults, consistent with males and females alike undergoing the breeding migration every year.

The model is also conditioned on Lexington Reservoir and inlet streams on the reservoir side of Alma Bridge Road being the only breeding source for this population and the adult newts in this population crossing Alma Bridge Road to breed (see Assumption 1 in section Materials and Methods). However, it is possible that there are adult newts in other upland areas around Lexington Reservoir that are breeding in the reservoir without crossing Alma Bridge Road. We suspect though that the number of newts in these other areas (i.e., areas south and west of Lexington Reservoir) is much lower than those in the upland east of Lexington Reservoir, on the other side of Alma Bridge Road, due to the relatively limited amount of upland habitat available to these newts for dispersal and refugia as a result of more residential development and Highway 17 in these areas. Also, there may be other breeding sources for this population besides Lexington Reservoir. For example, newts may breed in the upper reaches of Limekiln Creek (approximately 16 km of creek distance upstream of Alma Bridge Road) and Soda Springs Creek (approximately 19 km of creek distance upstream of Alma Bridge Road). If newts are breeding in Lexington Reservoir without crossing Alma Bridge Road or are also breeding in these other locations, annual recruitments from Lexington Reservoir or these other sources might be sustaining or supplementing the population, even though the high mortality rate of crossing Alma Bridge Road to breed in Lexington Reservoir would represent a population sink for the overall metapopulation. However, if these other breeding sources do exist, they may become less reliable in the future due to increased drought conditions as a result of climate change (Jones et al., 2017).

Assuming that all parameters and assumptions for the population viability model are correct, resulting in a possible extirpation of the local California newt population at Lexington Reservoir in 92 years, then reducing the roundtrip road-based mortality rate from the current 39.2 to 24.95% would allow the population to be sustained at approximately its current size after 132 years. These road-based mortality rates should therefore be considered when examining potential corrective measures to reduce the negative impact of this road-based mortality on this population.

Our population projections use our best estimates of the true survival rates of juveniles and adults in the Lexington Reservoir population in an average year given the available data. However, our juvenile survival rate might be less accurate than our adult survival rate. Adult California newts travelling to breeding sites have been observed during both daytime and nighttime hours, primarily during wet months of the year (Stebbins and McGinnis, 2012). In contrast, larvae of this species metamorphose and leave the aquatic breeding site for the terrestrial environment during the dry season (summer and fall; Kuchta, 2005). They likely travel at night, when temperatures and risk of desiccation are lowest. Traffic levels should also be lower at night (see section 8 of Supplementary material), resulting in lower risk of vehicular strike for juveniles attempting to cross Alma Bridge Road during the dry season. Dry season movement of juveniles appears to explain why we captured few juvenile California newts attempting to cross the road during the 2020/2021 wet season; juvenile captures accounted for only 9% of all California newt captures at the trap arrays. This runs counter to expectation, based on observations of another drift fence/pit fall trap study of California tiger salamanders in Alameda County (H. T. Harvey & Associates, 2004), where juveniles outnumbered adults in the captures, suggesting that the juveniles we captured represent only a small fraction of the total number of juveniles in the Lexington Reservoir population. The juveniles we captured metamorphosed the previous year and likely emerged from underground retreats to continue migrating away from the reservoir or forage for prey at the surface. Most juveniles in this cohort probably crossed the road in the previous dry season. In essence, we applied the reservoir-to-upland road mortality rate measured during the wet season to these dry-season crossings in preference to ignoring juvenile mortality. To our knowledge, no studies have directly measured the success rate of juvenile salamanders that attempt to cross a road during the dry season, but incorporation of these direct measurements would increase confidence in the parameterization of our model.

Another source of uncertainty in our population viability model is the estimate of the number of adults attempting to cross the road to breed. The actual number was likely somewhat higher. Data from carcass persistence trials conducted by the community scientists in the study area during the same breeding season[6] (accessed on February 8, 2022) suggest that some carcasses were removed by subsequent vehicle strikes, rain, or scavengers before they could be counted in the community scientist DOR surveys. Because these surveys were generally conducted twice per week (Parsons, 2021), carcass removal between surveys likely lead to underestimation of the true number of DOR in non-array road segments, underestimation of the total number of newts attempting to cross the road, and overestimation of time-to-extirpation. In contrast, our estimates of mortality rate for road-crossing attempts are largely robust to potential undercounting from carcass removal and imperfect searcher efficiency, because they were measured using array-based surveys, which were conducted every 24 h. Our examination of the carcass persistence results (M. Vonshak, unpublished data) suggests that the daily schedule allowed surveyors several chances to detect most carcasses.

Our study demonstrates how the population viability analysis can identify amphibian populations in danger of extirpation from direct mortality caused by vehicular strikes on roads. This technique may be particularly useful for elucidating population trajectory in non-intuitive situations where large populations undergo heavy losses of individuals on roads over long periods of time. We recommend that researchers undertaking these analyses account the roundtrip nature of (successful) adult migrations to and from breeding sites in situations where roads intervene between upland habitat and breeding sites. Furthermore, we recommend that researchers also incorporate potential vehicular strikes on juvenile life history stages (Petrovan and Schmidt, 2019), especially in light of evidence that mitigation efforts focused on juveniles can have large effects on population-level outcomes (Sterrett et al., 2018). Successful implementation of such relatively complex, including road mortality of multiple life history stages, models for diverse species in a variety of ecological situations will require demographic and natural history studies to obtain robust model parameters.

Summary and conclusion

In summary, based on the results of this study, over 13,700 adult California newts attempted to cross Alma Bridge Road during the 2020/2021 winter season in order to breed in Lexington Reservoir, experiencing a 39.2% roundtrip road-based mortality rate. Our population viability model indicates that this mortality of adults, combined with mortality of juveniles dispersing across the road following metamorphosis, is having a negative impact on the population using Lexington Reservoir to breed and if unmitigated

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[6] https://www.inaturalist.org/projects/pacific-newt-roadkill-longevity-study-2020-2021-lexington-reservoir

may cause this population to become extirpated in 92 years. This model-derived estimate of time-to-extirpation might be somewhat too short or too long, but is based on the best available data to inform the model parameters. Further investigation of demographic parameters for the Lexington Reservoir California newt population could be used to refine the model in the future. These investigations could include: (1) a mark-recapture component to more accurately determine the frequency of breeding attempts by males and females; (2) a detection probability factor based on the results of a carcass persistence study, as such a factor may influence both the overall road-based mortality rate and the population size in the model, and may direct future survey efforts to include more frequent surveys (e.g., twice a day); (3) an extended drift fence/pitfall trap array survey period throughout the year to capture newts dispersing during the non-breeding season, for example juvenile newts and non-migrating adult newts, to arrive at a more accurate road-based mortality rate for these life stages and seasons; and (4) multiple years of similarly procured capture and mortality data to reflect possible differences in numbers of newts migrating during drought years as opposed to years of average or above average rainfall, which would better estimate the adult breeding population.

It is likely that adult rough-skinned newts experienced similar rates of mortality during their migrations to and from the Lexington Reservoir breeding site. However, only 38 adult rough-skinned newts were observed in the traps compared to 2,054 adult California newts, indicating that the rough-skinned newt population breeding at the reservoir is far smaller than the corresponding California newt population, perhaps because this locality is near the southernmost limit of the rough-skinned newt's range. High levels of mortality on the road combined with a small population size might make the rough-skinned newt population more susceptible to potential extirpation in the near term than California newt. Although we did not estimate overall population size and mortality rates for rough-skinned newts due to the very low number of captures, such estimates based on a larger survey effort, combined with the same quantitative population modeling approach applied to the California newt population, may provide more information on the risk of extirpation to the rough-skinned newt population and how long it would take such extirpation to occur without intervention. Regardless, our study results indicate that temporary and permanent actions should be implemented to help preserve the local population of California newts, and these actions will also benefit rough-skinned newts.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because this study involved capture and release of live individuals in the field under a California Department of Fish and Wildlife Scientific Collection Permit No. S-200350006-20041-001.

Author contributions

JW developed the study design, supervised the surveys, analyzed the data, and wrote portions of the manuscript. JR analyzed the data, performed the statistical analysis, and wrote portions of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was partially funded by Regional Peninsula Open Space Trust and Midpeninsula Regional Open Space District.

Acknowledgments

The basis for this study was identified through the considerable efforts of Anne Parsons, surveying for road-killed newts along Alma Bridge Road and entering the data into iNaturalist. We would like to thank Blair Pagano, Dana Page, Jackson Bramham, Jared Bond, Mason Hyland, Michael Rhoades, and Vanessa Clayton of the Santa Clara County Parks Department for assisting with the installation of the drift fence/pitfall trap arrays and coordinating with the volunteers. We would also like to thank Anderson Wang, Ariel Starr, Chuck Wilson, Freya Prissberg, Joan Pinder, Kevin Yokoo, Marika Powers, Marisa Dobkins, Mojgan Mahdizadeh, Pam Hansen, Sadie Hine, Matt Bozzo, Shawn Lockwood, Thomas Wang, K. Perry, and Vanessa Roy, who, as volunteers, partnered with H. T. Harvey & Associates biologists Steve Carpenter, Matt Louder, Christian Knowlton, Will Lawton, Emily Malkauskas, Jane Lien, Craig Fosdick, Zach Hampson, Jazmine Jensen, and Stephen Peterson to conduct the daily surveys during the survey period. We would also like to thank Merav Vonshak of the BioBlitz Club and San Jose State University and her team of community scientists, particularly Robin Agarwal, who performed the surveys for newt mortalities along Alma Bridge Road and coordinated closely with our efforts during the 2020/2021 breeding season to ensure that our data were complementary rather than overlapping, inconsistent, or confounding. We would also like to thank Abra Kaiser for producing the maps. Finally, we would like to thank Neal Sharma of the Peninsula Open Space Trust, Julie Andersen, Brian Malone, Jane Mark, Tina Hugg, Alex Casbara, and Kirk Lenington of the Midpeninsula Open Space District, Harry Freitas and Ananth Prasad of Santa Clara County Roads and Airports, Jared Bond, Jeremy Farr of Santa Clara County Parks, Jae Abel and Shawn Lockwood of Valley Water, Merav Vonshak, Shani Kleinhaus of the Santa Clara Valley Audubon Society, Cheryl Brehme of the U.S. Geological Survey, Tiffany Yap of the Center for Biological Diversity, Fraser Shilling of the University of California at Davis Road Ecology Center, Terris Kasteen of the California Department of Fish and Wildlife, Dashiell Leeds of the Sierra Club, and Steve Rottenborn of H. T. Harvey & Associates for participating in the multiple discussions on the study, analytical methods, and our results and conclusions, and for providing comments on earlier drafts of this paper.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.944848/full#supplementary-material

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Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 07 February 2023
DOI 10.3389/fevo.2023.1007743

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 30 July 2022
ACCEPTED 18 January 2023
PUBLISHED 07 February 2023

CITATION
Tipton AF, Vázquez-Diosdado JA and
DeSantis DL (2023) Scale-dependent effects of
roadways on the movement behavior of a
large-bodied pit viper (*Crotalus horridus*).
Front. Ecol. Evol. 11:1007743.
doi: 10.3389/fevo.2023.1007743

Scale-dependent effects of roadways on the movement behavior of a large-bodied pit viper (*Crotalus horridus*)

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Roadways are among the most widespread and disruptive anthropogenic land use features that influence the behavior and movement of wildlife. Negative impacts of roadways have been well documented, but the behavioral impact of roadways on smaller, cryptic species has yet to be thoroughly examined. Using a novel integration of radio telemetry and tri-axial accelerometry, we evaluated the effects of roadways on the movement behavior of 26 adult Timber Rattlesnakes (*Crotalus horridus*) at coarse and fine time scales in central Georgia between June 2020 and November 2021. To interpret the effect of roads at a coarse time scale, we modeled the effect of mean annual distance to roadways (DTR) on annual measures of movement and space use by *C. horridus* using both radio telemetry and accelerometry derived metrics (RT and ACT metrics). To explore the fine-scale impact of roadways, we quantified RT and ACT metrics during confirmed road interactions (i.e., instances when individual snakes crossed a road or encountered a road but did not cross) and compared these instances to the RT and ACT metrics calculated across the remainder of the active season within this subset of snakes. Relating the annual RT and ACT metrics to DTR revealed no significant associations at a coarse time scale. However, the evaluation of *C. horridus* movement behavior during punctuated road encounters revealed that snakes increased RT and ACT metrics during the road interactions compared to metrics calculated across the remainder of the active season. This might indicate that the abundance of contiguous habitat adjacent to roadways at our study site is serving as an adequate buffer to any long-term shifts in movement behavior, but the potential hidden cost of increasing movement when snakes encounter roads could have negative implications for populations that encounter roads more frequently, even in the absence of significant direct road mortality. Overall, integrating radio telemetry and accelerometry and adopting a scale-dependent approach to quantifying movement allowed for a more detailed evaluation of the response of *C. horridus* to roadways. This approach holds promise for detecting and interpreting previously overlooked short-term alterations in snake movement behavior with potentially significant fitness consequences.

KEYWORDS

road ecology, accelerometry, machine learning, radio telemetry, home range

Introduction

In an increasingly human-modified world, it is essential to understand how organisms respond to anthropogenic activities and disturbances. Among the multitude of human effects on natural landscapes, the historic and ongoing construction of roadways is viewed as a leading disruptor of wildlife behavior and ecology globally (Oxley et al., 1974; Lovallo and Anderson, 1996; Trombulak and Frissell, 2000; Underhill and Angold, 2000; Spellerberg, 2002; Forman et al., 2003; Jaeger et al., 2005; Reynolds-Hogland and Mitchell, 2007; Fahrig and Rytwinski, 2009). Roads negatively impact wildlife most directly through vehicle-induced mortality (Ashley and Robinson, 1996; Shepard et al., 2008a; Quintero-Ángel et al., 2012; Rytwinski and Fahrig, 2012), but sub-lethal effects of roads have also been well documented. For example, by fragmenting habitat and acting as potential barriers to movement, roadways can limit gene flow and reduce population viability (Lodé, 2000; Keller and Largiader, 2003; Shine et al., 2004; Shepard et al., 2008b; Clark et al., 2010; Holderegger and Di Giulio, 2010). Additionally, the increased human presence, vehicular traffic, and noise associated with roads can lead to maladaptive behaviors and reduced fitness for individuals (Ware et al., 2015; Ng et al., 2019; Zhou et al., 2020). The extensive edge habitat associated with roadways can also attract some wildlife, creating a classic ecological trap for those species drawn to edges for foraging, nesting, or thermoregulating (Cowardin et al., 1985; Aresco, 2005; Mata et al., 2017). Ultimately, the alteration of behavior is at the core of wildlife-road interactions (Lomas et al., 2019), with variations in patterns of animal movement and space use in relation to roadways being a strong indicator of the relative risk that these features pose to individual species and populations.

Animals move through their environments in efforts to locate and acquire critical resources such as food, water, and mating partners, while minimizing potentially harmful encounters with predators, competitors, or unfavorable environmental conditions (Nathan et al., 2008). Accordingly, disrupting an animal's movement behavior can have significant fitness effects. However, numerous intrinsic and extrinsic factors simultaneously influence movement decisions, making it difficult to pinpoint the mechanistic roles of each individual driver. Among these, an individual's sex, age, and motivational state (e.g., behavioral season) have all been demonstrated to influence movement patterns, and, consequently, are important considerations when exploring the effects of individual extrinsic factors, such as roadways, on movement patterns (Waldron et al., 2006; Petersen et al., 2019). Unsurprisingly, species-specific behavioral responses to roadways can vary tremendously, ranging from avoidance (Thurber et al., 1994; Rondinini and Doncaster, 2002; Andrews et al., 2008; McGregor et al., 2008; Hibbitts et al., 2017) to strong attractance (Mumme et al., 2000; Aresco, 2005). For predators, the increase in concentrations of prey adjacent to roads has been suggested to attract some species and alter their patterns of space use (Barrientos and Bolonio, 2009; Leblond et al., 2013; Ruiz-Capillas et al., 2013). Road surface, road width, and traffic volume are also important factors that can produce variation within and between species (Ware et al., 2015; Hibbitts et al., 2017; Ng et al., 2019). Even further, the spatial and temporal scale at which movement is measured can have pronounced impacts on inferred responses, especially if an individual's interactions with roadways are infrequent, which might be the case for those species that tend to avoid roads (Shepard et al., 2008b; Hibbitts et al., 2017). Clearly, careful selection of study organisms and suitable methods for quantifying movement is required for a detailed and accurate understanding of species-specific responses.

Despite the aforementioned importance of evaluating the relationships between roads, intrinsic variables, and movement behavior across a diversity of wildlife, such explorations are limited by the available methods for monitoring movement and space use in nature. For many small-bodied and secretive taxa, such as snakes, these constraints are amplified, as collecting movement data across a large sample of individuals and over meaningful time frames has been historically challenging or impossible. As such, manipulative experiments and predictive simulations with longitudinal datasets have represented important tools for evaluating the impact of roads on snake movement behavior. From the few manipulative studies conducted, researchers found that road crossing propensity differed between snake taxa with smaller bodied species exhibiting higher road avoidance than larger species (Andrews and Gibbons, 2005). They also found that pit vipers (Crotalinae) crossed roads more slowly compared to other species (Colubridae) (Andrews and Gibbons, 2005). Garter Snakes (*Thamnophis sirtalis parietalis*) also displayed avoidance behavior when placed along roads, and the ability of mate-searching males to follow female pheromone trails decreased across the road surface compared to surrounding habitat (Shine et al., 2004). Additionally, road crossing probabilities of Brown Treesnakes (*Boiga irregularis*) were found to decrease with increasing road magnitude but increased with increasing body size and humidity (Siers et al., 2016). Road avoidance behavior was also revealed through predictive simulations with longitudinal datasets. Eastern Massasauga Rattlesnakes (*Sistrurus catenatus*), Eastern Hognose Snakes (*Heterodon platyrhinos*) and Brown Treesnakes (*Boiga irregularis*) were found to cross roads significantly less frequently than expected (Shepard et al., 2008b; Robson and Blouin-Demers, 2013; Siers et al., 2014), and further, *H. platyrhinos* avoided crossing paved but not unpaved roads (Robson and Blouin-Demers, 2013). Although both the manipulative studies and predictive simulations report informative findings on the movement response of snakes to roadways, considerable paucity remains in our understanding of how individual species respond behaviorally to roads in a natural setting and how these responses might vary relative to key factors such as an individual's sex or motivational state, or the spatial and temporal scales considered.

Historically, using radio telemetry to monitor a subset of individuals and estimating the total extent of space used (i.e., home range size) has represented the best approach for quantifying the long-term behavioral responses of snakes to roadways and other prominent landscape features (Clark et al., 2010; Kapfer et al., 2010; Row et al., 2012; Ettling et al., 2013; Anguiano and Diffendorfer, 2015). However, the estimation of an animal's home range size and movement distances largely ignores the fine-scale temporal dimensions of animal movement, as individuals can potentially alter the frequency and duration of movements independent of spatial shifts. The recent explosion of biologging technologies has afforded researchers with an expanded toolkit for measuring the movement behavior of organisms in the field. Among these, accelerometer dataloggers are becoming increasingly popular in field studies of wildlife behavior (Brown et al., 2013; Wilmers et al., 2015). When paired with advanced machine learning techniques, accurate and automated classification of discrete behaviors can be achieved using animal-borne accelerometer data. However, until very recently, accelerometers and other biologger applications were mostly restricted to larger terrestrial mammals, large birds, and aquatic vertebrates (Brown et al., 2013). The validation of accelerometer monitoring of movement behavior in rattlesnakes has now set the stage for applied extensions of these techniques in hypothesis-testing frameworks (DeSantis et al., 2020). Herein, we report on an integration of radio telemetry and accelerometry allowing for the evaluation of movement responses by Timber Rattlesnakes (*Crotalus horridus*) to roadways in the Piedmont ecoregion of Georgia, USA, where remaining *C. horridus* populations are suspected to be in decline (Jenkins et al., 2021). We aimed to evaluate the influence of roads on the spatial movement patterns and daily movement durations of *C. horridus* at both a coarse time scale (annual and seasonal) and fine time scale (during isolated road interactions) while also considering the potential effects of sex and behavioral season (non-mating, mating) in these relationships. Preliminary observations of telemetered *C. horridus* seemingly decreasing movement in close proximity to roadways suggested that roadways may be acting as a semipermeable barrier to movement. As such, we hypothesized that snakes that are, on average, closer to roads will constrict their movements and thus exhibit smaller home range and movement estimates. Further, we hypothesized that snakes would decrease movement during isolated road encounters compared to time periods in which they were not encountering roads.

Materials and methods

Study system

The field study site is located in Putnam County, Georgia, within the Cedar Creek Wildlife Management Area (CCWMA) and Oconee National Forest (ONF) (centered on N 33°14′16.33” W 83°30′48.24″). The ONF is mixed use, with a combination of public and private land. The habitat is characteristic of the Piedmont ecoregion in Georgia, consisting mainly of upland Loblolly Pine (*Pinus taeda*) and riparian hardwood forest and a mixture of the two where they adjoin. A key feature of the study site is the high levels of habitat heterogeneity therein, with clear-cut fields, uneven stand ages, residential properties, and an abundance of paved and unpaved roads all present within the study area. The paved roads present at the site are moderately trafficked and are eight meters wide. Hillsboro Road and Stanfordville Road, the paved roads that somewhat serve as the northern and eastern borders of our study area, respectively, have an approximate traffic volume of 122 vehicles per day (Georgia Department of Transportation, 2022). Highway 212 largely acts as the southern border of our study site and has a traffic volume of approximately 1,226 vehicles per day (Georgia Department of Transportation, 2022). The unpaved, dirt and gravel roads at the site vary between approximately six and eight meters wide. These roads are low-use with estimated traffic volumes not typically exceeding 20 vehicles per day (personal observation). The traffic volumes likely increase during popular hunting seasons (late fall to winter/early spring), but these times do not substantially overlap with the snakes' active season.

Timber Rattlesnakes (*C. horridus*) are heavy-bodied pit vipers historically distributed throughout eastern North America (Martin et al., 2008). They are forest specialists and ambush predators, with rodents and lagomorphs making up the majority of their diet (Martin et al., 2021). As a result of local environmental pressures and the genetic distance between populations (Bushar et al., 1998), considerable size variation is noted throughout the range of *C. horridus*, but male-biased sexual size dimorphism is ubiquitous (Stengle et al., 2021). Although locally abundant at our study site, *C. horridus* have a patchy distribution throughout the Piedmont ecoregion of Georgia (Jenkins et al., 2021). Much of what is known of *C. horridus* ecology and behavior is from studies conducted on populations in the northern portion of their distribution, with a relative dearth of research from the southern regions. Populations in the north have seen declines due to road mortality, reduced gene flow, and disease, which are likely threats to populations in the south, as well (Petersen and Sealy, 2021). Life history traits of *C. horridus* (i.e., long lived, long gestation periods, habitat specialists) make them especially vulnerable to the lethal and sub-lethal effects of roads (Fahrig, 2007), so much that roads are identified as one of the leading threats to range-wide population viability (Petersen and Sealy, 2021).

Field data collection

Between June 2020 and November 2021, we employed radio telemetry and accelerometry monitoring of 26 adult Timber Rattlesnakes (RT – Female: $N = 14$, Male: $N = 12$; ACT – Female: $N = 10$, Male: $N = 8$; Tipton, 2022). One female was radiotracked during the study period but was not included in statistical analyses due to being confirmed as gravid. One male was lost as a result of suspected road mortality or transmitter failure as the snake remained within close proximity of a roadway for a month (8 relocations) before we lost the signal. This male only contributed data to our RT spatial analyses as the ACT was not retrieved. Additional snakes that were equipped with ACTs contributed to the RT analyses but not the ACT analyses due to ACT malfunctions (two females, one male). One additional male was lost to suspected transmitter failure, and another male was lost to suspected predation shortly after monitoring began; neither were included in RT or ACT analyses.

Radio transmitters (Holohil Systems Ltd., Model SB-2 T) and ACTs (Technosmart Europe srl., AXY-5) were coupled, internally implanted (Reinert and Cundall, 1982), and securely sutured to a rib (Hardy and Greene, 1999, 2000) in the same position and orientation for all individuals. Implants (RT = 5 g; ACT = 10 g) comprised ≤3% of each individual's body mass at the time of implantation (Table 1). RTs had a battery life of 10 months, and ACTs had 1 Gb of storage capacity allowing for approximately 10 months of continuous, low frequency (1 Hz) recording. Rattlesnakes were released at the original site of the capture within 3 days of implantation of the RT-ACT device and were relocated every 3–4 days during the active season (March–November) and biweekly during the inactive season (December–February) to collect RT derived spatial movement data. Detailed behavioral observations were recorded during relocations along with environmental conditions, habitat type, and geographic coordinates with a hand-held GPS (Garmin Oregon 700, accuracy ≤5 m).

Accelerometer data processing, model validation, and application

We applied the ACT data processing protocols developed and validated for rattlesnakes (*C. atrox*) by DeSantis et al. (2020) to our remotely collected ACT data in *C. horridus*. During each RT data collection trip (every 3–4 days), rattlesnakes were observed for visual field validation of ACT signals corresponding to our two broad behavioral categories, "immobile" and "moving" (Le Roux et al., 2017; Barwick et al., 2018; DeSantis et al., 2020). The behavioral category "moving" was characterized by outstretched, full body motion, while "immobile" was defined as a lack of full body motion regardless of posture. Detailed and timestamped notes along with video recordings were taken during observations of ACT-equipped individuals in the field. These notes and videos were used as a reference during the manual annotation of behaviors within ACT datasets. These time-matched observations of behavior in the field represented the training and testing data for the ensemble learning model.

TABLE 1 Snake ID, sex, Snout-Vent Length (SVL), weight, RT monitoring durations, and ACT monitoring durations for all *Crotalus horridus* monitored.

Snake ID	Sex	SVL (mm)	Weight (g)	RT monitoring duration	ACT monitoring duration	Mean distance to road
CRHO_01	f	690	267	7/29/20–10/27/20	7/29/20–10/27/20	52.53
CRHO_02	f	800	320	8/21/20–10/17/20	8/21/20–10/17/20	135.89
CRHO_03	f	905	585	8/7/20–10/26/21	8/7/20–5/21/21	490.90
CRHO_04	f	925	600	6/25/21–11/9/21	N/A	110.44
CRHO_05	f	930	587	10/13/20–10/29/21	**10/13/20–6/25/21	281.12
CRHO_06	f	980	811	7/17/20–10/12/21	7/17/20–10/12/21	200.81
CRHO_07	f	1,030	847	7/23/21–10/22/21	N/A	176.82
CRHO_08	f	1,100	1,300	5/28/21–11/9/21	5/28/21–9/24/21	415.86
CRHO_09	f	1,108	1,200	9/11/20–10/29/21	N/A	341.39
CRHO_10	f	1,140	1,562	11/6/21–6/15/21	**11/6/20–6/3/21	911.68
CRHO_11	f	1,165	856	9/3/21–11/12/21	N/A	371.87
CRHO_12	f	1,195	1,299	9/1/20–7/7/21	9/1/20–8/3/21	287.26
CRHO_13	f	1,200	1,278	11/6/20–10/15/21	4/9/21–10/15/21	379.45
CRHO_14	f	1,250	1,371	5/21/21–10/22/21	N/A	419.37
CRHO_15	m	820	457	6/10/20–9/28/21	6/10/20–10/28/20	264.57
CRHO_16	m	825	470	6/10/20–5/11/21	6/10/20–10/30/20	257.17
CRHO_17	m	950	646	8/3/21–11/2/21	N/A	481.56
CRHO_18	m	1,050	872	8/18/20–9/25/20	**8/18/20–9/25/20	140.07
CRHO_19	m	1,100	1,494	8/24/21–10/15/21	N/A	268.92
CRHO_20	m	1,140	1,217	6/10/20–9/21/21	6/10/20–9/21/21	251.84
CRHO_21	m	1,140	1,255	6/10/20–11/5/21	**6/10/20–10/27/20	359.36
CRHO_22	m	1,190	1,557	*8/18/20–9/11/20	**8/18/20–9/11/20	N/A
CRHO_23	m	1,200	1745	8/7/20–11/9/21	8/7/20–5/21/21	554.70
CRHO_24	m	1,270	1926	9/17/21–10/26/21	N/A	291.53
CRHO_25	m	1,300	1799	*4/30/21–5/21/21	*4/30/21–5/21/21	N/A
CRHO_26	m	1,340	1700	10/8/21– *present*	N/A	712.25

*Data is not included in analyses because of insufficient monitoring duration. **Not included due to lost/malfunctioning ACT.

A total of 40 descriptive statistics were computed for the magnitude of acceleration, defined as $A = \sqrt{A_X^2 + A_Y^2 + A_Z^2}$, and across the *x* (surge; forward and backward motion), *y* (sway; side-to-side motion), and *z* (heave; upward and downward motion) ACT axes. These descriptive statistics were calculated to summarize acceleration data and identify relationships between ACT signals and behavioral category. The following features were computed for overall dynamic acceleration and acceleration along each individual axis ($\vec{A}, A_X, A_Y, A_Z$): mean, standard deviation, interquartile range, maximum, minimum, mean of the difference between consecutive points, standard deviation of the difference between consecutive points, interquartile range of the difference between consecutive points, maximum of the difference between consecutive points, and minimum of the difference between consecutive points. These summary stats were initially calculated to be "rolling" over varying temporal window sizes (4, 5, 10, 30, and 60-s period basis) to identify the optimal scale at which to classify activity with machine learning models. Summary statistics were ranked using ReliefF (Farzaneh, 2022) feature selection for the classification of immobile and moving behaviors.

An AdaBoost ensemble learning algorithm (Rokach, 2010) was implemented using the fitcensemble function in MATLAB (2019). The AdaBoost algorithm learner was set to have a maximum number of splits of 512 and a minimum leaf size of five. Classification performance was evaluated using a fivefold cross validation technique (Kononenko, 1994). The initial dataset was split into five subsets of equal size and five iterations were performed. Within this technique at each iteration, four of the subsets were used to train the algorithm and the remaining one was used as a test set. This process was repeated five times, each time changing the training and test subsets until all subsets have been used as a test. Performance values are computed for the test set across each iteration. Following model training and testing, the validated model was applied to the full field-collected datasets for automated behavioral classification. Using these behavioral predictions, continuous activity budgets (time classified as moving vs. immobile) were produced enabling quantification of real-time movement durations across various time scales (daily, weekly, monthly) for each individual.

Radio telemetry and accelerometry movement metrics

Spatial aspects of rattlesnake movement were quantified for each individual within the non-mating (March–July) and mating

(August–November) seasons using the following radio telemetry derived metrics: Meters Per Day (MPD), calculated as the sum of the straight-line distances between successive relocation points for an individual divided by the total number of days monitored, Distance Per Movement (DPM), calculated as the mean straight-line distance between relocation points that were ≥ 5 m apart, Minimum Movement Frequency (MMF), calculated as the number of movements (≥5 m) made by an individual out of N relocations in a specifically defined time period, and Motion Variance (MV) extracted from individual dynamic Brownian Bridge Motion Models. Motion Variance estimated the variance among displacement distances within a moving window of nine radio telemetry relocations (i.e., 1 month increments of spatial data). Higher MV values indicate more variable or abnormal movement relative to the sampling duration considered (Kranstauber et al., 2012; Silva et al., 2018). Annual and seasonal home range measurements were calculated for each individual with commonly used home range estimators, including the 100% Minimum Convex Polygon (MCP) (Row and Blouin-Demers, 2006) and 95 and 50% fixed-kernel Utilization Distributions (UD) with the plug-in bandwidth matrix (Bauder et al., 2015, 2016). The plug-in bandwidth matrix for the UD home ranges was chosen because it has been demonstrated to be robust to variation in sampling rate and duration (Bauder et al., 2015). RT movement metrics and home range sizes were calculated in R using the *adehabitat*, *adehabitatHR,* and *adehabitatLT* packages (R Core Team, 2022).

Temporal aspects of rattlesnake movement were quantified for each individual annually as well as within the non-mating and mating seasons. Accelerometry derived movement metrics included mean time spent moving per 24 h (Mov24) and mean number of movement bouts (periods of movement ≥60-s) per 24 h (Bout24). In order to evaluate the effects of roadways on RT and ACT movement metrics, ArcGIS was used to calculate the Euclidean distance (m) to the nearest roadway for each relocation point. The mean distance to roadway (DTR) for each individual was quantified both annually and within the non-mating and mating season.

Statistical analysis: Coarse scale effect of DTR on annual RT and ACT movement metrics

Linear Mixed Effects (LME) models were employed to test for the effects of sex, behavioral season, and DTR on movement and space use patterns. Response variables (RT and ACT movement metrics and home range estimations) were modeled with separate LME models to evaluate significant effects and interactions. Rattlesnake ID was modeled as a random effect to control for non-independence of data across time. Fixed effects included sex (male, female), behavioral season (non-mating, mating), and DTR. Our RT response variables included MPD, DPM, MMF, MV, 100% MCP, 95% UD, and 50% UD and the ACT response variables included Mov24 and Bout24. Appropriate transformations for each response variable were determined and employed in the model. This resulted in the following set of response variable transformations: MPD (log base 10), DPM (log base 10), 100% MCP (log base 10), 95% UD (log base 10), 50% UD (square root), Mov24 (log base 10). Model fit was evaluated with marginal and conditional pseudo-R^2 measures. Marginal pseudo-R^2 describes the proportion of model variance attributed to the fixed effects, while conditional pseudo-R^2 describes the proportion of model variance attributed to both fixed and random effects. The emmeans package in R (Lenth, 2022) was used for *post hoc* evaluation of relationships between variables when significant main effects were detected in the LME models.

Fine-scale road interactions

Along with evaluating the effect of roads on coarser scale measures of movement (annual and seasonal RT and ACT metrics), we also evaluated the movement response of snakes to roadways during confirmed road encounters. We investigated this direct movement response of *C. horridus* to roads by identifying snakes within our sample with confirmed road interactions during the monitoring period (i.e., instances when individual snakes either crossed a road or encountered a road but did not cross and retreated). To evaluate whether the movement behavior of these specific individuals differed during these brief events relative to periods when they were not encountering roadways, we calculated RT movement metrics (MPD, DPM) and ACT movement metrics (Mov24, Bout24) across the four relocations (2 weeks) preceding each road encounter for comparison with the same metrics calculated from all remaining relocations within the datasets for this subset of individuals. We opted for a fixed temporal window for the fine-scale movement analysis to standardize the amount of data contributed across individuals. We selected four relocations, or 2 weeks for ACT data, as it represented the mean and median value for the number of relocations (range: 1–9 consecutive relocations within 25 m of roadway) associated with each of the 17 road encounters observed during the monitoring period. These RT and ACT metrics were then pooled across all snakes that interacted with roads into their respective categories (road interaction and no road interaction) for analysis. Non-parametric Mann–Whitney *U* tests were conducted to compare the mean RT and ACT movement metrics (MPD, DPM; Mov24, Bout24) between the road interaction and no road interaction categories.

Results

RT field deployment

Individual rattlesnakes were radiotracked for durations ranging from 35 to 503 days (mean ± SD = 237 ± 169) between June 2020 and November 2021 for a cumulative total of 1,437 telemetry relocations (mean ± SD = 60 ± 40; Table 1). Male (*N* = 14) and female (*N* = 17) annual movement and space use measures were calculated and condensed into behavioral season (non-mating, mating) for analyses. In total, 47 RT snake-season data points (17 non-mating, 30 mating) were accumulated. The mean annual MPD (±SD) for males was 31.5 ± 15.9 and 18.7 ± 7.00 m for females. The mean annual DPM for males was 123.1 ± 58.6 and 76.0 ± 24.0 m for females. Mean annual MMF for males was 0.79 ± 0.12 and 0.81 ± 0.11 for females. The mean annual MV for males was 2.62 ± 2.39 and 0.92 ± 0.75 for females. Mean annual 95% UD for males was 44.0 ± 33.0 ha and 23.5 ± 15.2 ha for females. The mean annual 50% UD for males was 6.55 ± 6.4 ha and 3.59 ± 3.43 ha for females. Mean annual 100% MCP for males was 28.2 ± 25.4 and 13.5 ± 10.1 for females (Figure 1).

ACT field deployment and model validation

Individual rattlesnakes were monitored *via* accelerometry for durations ranging from 57 to 450 days (mean ± SD = 233 ± 130) for a total

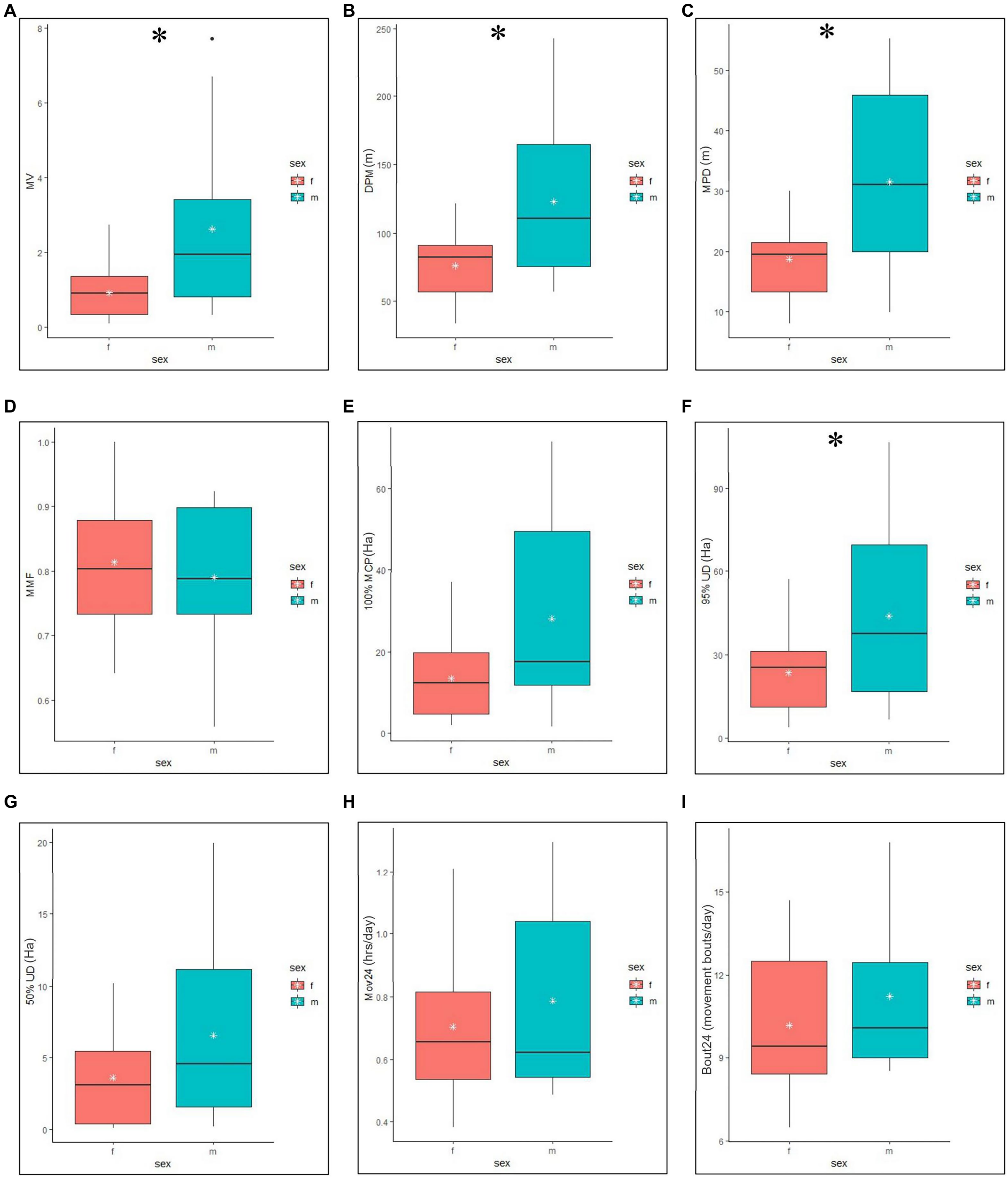

FIGURE 1
Annual minimum, median, mean (white stars), and maximum values, and the interquartile ranges of male and female *Crotalus horridus* RT and ACT metrics. RT derived movement measures **(A–D)** include Motion Variance (MV), Distance Per Movement (DPM), Meters Per Day (MPD), and Minimum Movement Frequency (MMF). RT derived space use measures **(E–G)** include 100% Minimum Convex Polygons home range (MCP), 95% fixed kernel Utilization Distribution home range (95% UD) and 50% fixed kernel Utilization Distribution core use area (50% UD). ACT derived movement measures **(H,I)** include the mean time spent moving per 24h (Mov24) and the mean number of movement bouts per 24h (Bout24). Asterisks denote significant differences ($p<0.05$).

of 2,791 recording days between June 2020 and November 2021 (Table 1). Male ($N=4$) and female ($N=7$) annual movement durations were calculated and condensed into behavioral season (non-mating, mating) for analyses. In total, 25 ACT snake-season data points (12 non-mating, 13 mating) were accumulated. The mean annual Mov24 (±SD) for males was 0.78 ± 0.35 h and 0.70 ± 0.27 h for females. Mean annual Bout24 for males was 11.2 ± 3.2 bouts and 10.2 ± 2.9 bouts for females.

The optimal window size for classifying immobile and moving behaviors was determined to be 60 s. The initial validation dataset contained a total of 828 60-s (13.80 h) observations of moving and immobile behaviors. Six hundred and sixty of the 60 s observations were of immobile behavior (11 h) and 168 of the 60 s observations were of moving behavior (2.80 h). At a 60 s window size, the model accurately classified 98.79% of both immobile and moving behaviors. The model classified the immobile behaviors with a precision of 98.95%, a recall of 99.55%, an F-score of 99.24%, and a specificity of 95.83%. The model classified moving behavior within the field datasets with a precision of 98.17%, a recall of 95.83%, an F-score of 96.99%, and a specificity of 99.55%.

Linear mixed effects models: Annual RT movement metrics

Preliminary LME models included sex, season, and distance to road (DTR) as fixed effects, and our spatial response variables included MPD, DPM, MMF, MV, 100% MCP, 95% UD, and 50% UD. Limited within-season sample sizes resulted in a failure to converge across most models when including snake ID as a random effect. As a result, we calculated annual RT metrics, and the final spatial models include only sex and DTR as fixed effects.

In the annual LME models with RT response variables, there was a significant main effect of sex on MPD [Effect Size (ES) = 0.83 ± 0.39 SE, *p* = 0.04; Table 2] detected, with males moving more per day than females. *Post hoc* estimated marginal means pairwise comparisons also detected a significant difference in mean annual MPD between males and females ($t_{18.4} = -2.467$, *p* = 0.0237). There was no significant effect of DTR on MPD. The marginal and conditional pseudo-R^2 measures of fit were 0.24 and 0.44 (Table 3). A significant main effect of sex was detected on DPM (ES = 0.74 ± 0.33 SE, *p* = 0.04), with males moving further per movement compared to monitored females. *Post hoc* estimated marginal means pairwise comparisons also detected a significant difference in mean annual DPM between males and females ($t_{18.8} = -2.694$, *p* = 0.0144). There was no main effect of DTR on DPM. The marginal and conditional pseudo-R^2 measures of fit were 0.27 and 0.52. No significant main effect of sex or DTR was detected on MMF. The marginal and conditional pseudo-R^2 measures of fit were 0.003 and 0.46. A significant main effect of sex was detected on MV (ES = 2.33 ± 0.82 SE, *p* = 0.009), with males having a higher measure of MV compared to monitored females. *Post hoc* estimated marginal means pairwise comparisons also detected a significant difference in mean annual MV between males and females ($t_{17.6} = -2.613$, *p* = 0.0178). There was no main effect of DTR on MV. The marginal and conditional pseudo-R^2 measures of fit were 0.29 and 0.39. There was no significant main effect of sex or DTR on 100% MCP. The marginal and conditional pseudo-R^2 measures of fit were 0.12 and 0.51. A significant main effect of sex was detected on 95% UD (ES = 1.58 ± 0.69 SE, *p* = 0.03) with male home range sizes being larger in comparison to females, but the estimated marginal means *post hoc* pairwise comparison of male and female 95% UD did not detect a significant difference ($t_{18.7} = -1.561$, *p* = 0.1352). The marginal and conditional pseudo-R^2 measures of fit were 0.19 and 0.46. No significant main effect of sex or DTR was detected on 50% UD (Table 2). The marginal and conditional pseudo-R^2 measures of fit were 0.11 and 0.28 (Table 3).

TABLE 2 Coefficients, standard error, and *p*-values for individual RT derived annual movement and space use model parameters.

Model parameter	Coefficient	SE	*p*
Meters Per Day			
Sex (Male)	0.83	0.39	0.04*
DTR	0.0009	0.0006	0.15
Sex*DTR	−0.001	0.001	0.30
Distance Per Movement			
Sex (Male)	0.74	0.33	0.04*
DTR	0.0007	0.0005	0.18
Sex*DTR	−0.0009	0.0008	0.31
Minimum Movement Frequency			
Sex (Male)	−0.02	0.09	0.85
DTR	0.0002	0.0001	0.22
Sex*DTR	−0.00002	0.0002	0.93
Motion Variance			
Sex (Male)	2.33	0.82	0.009*
DTR	0.002	0.001	0.08
Sex*DTR	−0.004	0.002	0.08
100% Minimum Convex Polygon			
Sex (Male)	1.68	0.85	0.06
DTR	0.002	0.001	0.26
Sex*DTR	−0.003	0.002	0.12
95% Utilization Distribution			
Sex (Male)	1.58	0.69	0.03*
DTR	0.002	0.001	0.09
Sex*DTR	−0.003	0.002	0.09
50% Utilization Distribution			
Sex (Male)	1.58	0.98	0.12
DTR	0.002	0.002	0.19
Sex*DTR	−0.003	0.002	0.23

Reference levels for parameters are in parentheses (Male), and coefficients can be used to interpret the direction of individual effects. Asterisks between model parameters denote a tested variable interaction. Asterisks next to *p* values denote statistical significance.

TABLE 3 Marginal and conditional R^2 for individual RT derived annual movement LME models.

Model	R^2M	R^2C
Minimum Movement Frequency	0.003	0.456
100% Minimum Convex Polygon	0.124	0.506
Minimum Movement Frequency	0.079	0.315
50% Utilization Distribution	0.109	0.284
Meters Per Day	0.243	0.441
Distance Per Movement	0.265	0.523
95% Utilization Distribution	0.187	0.460
Motion Variance	0.286	0.386

Linear mixed effects models: Annual ACT movement metrics

Limited within-season and within-sex sample sizes resulted in a failure to converge across our ACT movement models. As a result, we calculated annual ACT movement metrics (Mov24 and Bout24) for our full sample of ACT-monitored snakes (males and females pooled) in order to evaluate the relationship between DTR and ACT derived movement durations. In the annual LME models with ACT response variables, there was no significant main effect of DTR detected on Mov24 or Bout24 for this pooled sample (Table 4). The marginal and conditional pseudo-R2 measures of fit for the Mov24 and Bout24 models were 0.0003 and 0.53 and 0.016 and 0.44, respectively (Table 5).

Fine-scale road interactions

Out of our full RT and ACT monitored sample, nine individuals (RT: five females, four males; ACT: three females, two males) were observed interacting with roads by either a confirmed crossing event or approaching within 25 m of a road and retreating. There were a total of 17 confirmed road interactions resulting in 15 road crossings and two instances of a snake approaching a road and retreating. The majority of confirmed road encounters resulted in a crossing event (88%).

There was a significant difference detected between the mean Mov24 for the two categories, road interaction and no road interaction ($W = 23{,}516$, $r = 0.10$, $p = 0.007$), with Mov24 (mean ± SD = 1.04 ± 1.6) during the road interactions being significantly higher than Mov24 (mean ± SD = 0.65 ± 0.98) when snakes were not interacting with a road (Table 6 and Figure 2). There was also a significant difference detected between the two categories for MPD ($W = 6{,}313$, $r = 0.14$, $p = 0.007$), with MPD during the road interactions (mean ± SD = 83.0 ± 71.8) being significantly higher than MPD when snakes were not interacting with a road (mean ± SD = 67.3 ± 95.7). No significant difference was detected in DPM or Bout24 between the road interaction and no road interaction categories (Table 6 and Figure 2).

Discussion

Historically, behavioral field monitoring of small and secretive animals has been methodologically challenging, limiting the ability to make comprehensive evaluations of the effects of anthropogenic landscape features, such as roadways, on movement patterns. With the recently validated RT-ACT framework for use in pit vipers, we sought to better understand the behavioral response of Timber Rattlesnakes (*Crotalus horridus*) to roadways. Based on preliminary observations, we hypothesized that roadways would constrain RT and ACT movement metrics at both a coarse and fine time scale, but our results did not support this prediction. The evaluation of RT metrics and ACT metrics at a coarse, annual time scale did not reveal any significant effect of the annual mean distance to roadways. However, a fine-scale analysis comparing the RT and ACT movement metrics during confirmed road interactions to metrics across all remaining relocations revealed a significant increase in movement distances and durations by *C. horridus* during these brief encounters with roadways. Although the individuals included in our sample did not interact with roads as frequently as expected given the high concentration of roadways at the site (Figure 3), this hidden fine-scale variation in movement could carry significant behavioral implications in populations where individuals more frequently interact with roads. These results also feature the importance of considering multiple time scales when evaluating the behavioral response in question and demonstrate the utility of accelerometry in longitudinal studies of pit viper movement behavior.

TABLE 4 Coefficients, standard error, and *p*-values for ACT derived annual movement model parameters.

Model parameter	Coefficient	SE	*p*
Mean Time Spent Moving per 24 h			
DTR	0.0002	0.0004	0.66
Mean Number of Movement Bouts per 24 h			
DTR	−0.0003	0.004	0.95

Coefficients can be used to interpret the direction of individual effects.

TABLE 5 Marginal and conditional R^2 for individual ACT derived annual movement LME models.

Model	R^2M	R^2C
Mean Number of Movement Bouts per 24 h	0.016	0.438
Mean Time Spent Moving per 24 h	0.0003	0.526

TABLE 6 Mann–Whitney *U* test comparing response variables (MPD, DPM, Mov24, and Bout24) between road interactions and no road interactions.

Response variable	*W*	*r*	*p*
MPD	6,313	0.14	0.007
DPM	5,099	0.11	0.07
Mov24	23,516	0.10	0.007
Bout24	25,948	0.05	0.18

Effect of roads on annual RT and ACT movement measures

Mean DTR did not affect our RT and ACT response variables when controlling for sex, indicating that at an annual time scale, the distance to roads does not significantly contribute to variation in individual movement patterns. Similar studies have found that human disturbance does not significantly change the spatial movement patterns of snakes (Shepard et al., 2008b; Lomas et al., 2019; Carrasco-Harris et al., 2020), likely due to the presence of contiguous habitat adjacent to the disturbance (Nordberg et al., 2021). Although roads are prominent features at our study site, there is sufficient unfragmented habitat alongside of the roads that likely serves as a buffer to any long-term shifts in movement behavior and space use of *C. horridus* (Figure 3). Additionally, the life history traits associated with habitat specialists, such as *C. horridus* (i.e., adapted to contiguous habitat with abundant cover), could result in a reduced boundary response given the relatively low encounter rate with habitat boundaries, such as roads (Fahrig, 2007). This could explain the lack of an annual spatial response in our dataset, given that roads at our site do not appear to be acting as impermeable barriers to movement, as 15 of 17 road interactions resulted in confirmed road crossing events. The low frequency of road interactions observed within our monitored sample of snakes, despite many individuals occupying home ranges in relatively close proximity to roadways (mean DTR = 342 m; see Table 1), also corresponds with previous observations of general road avoidance by snakes (Shine et al., 2004; Andrews et al., 2008; Shepard et al., 2008b; Robson and Blouin-Demers, 2013).

The absence of an effect of DTR on the annual RT and ACT movement metrics of *C. horridus* could also, in part, be explained by strong site fidelity (Marshall et al., 2020; Nordberg et al., 2021). Pit vipers

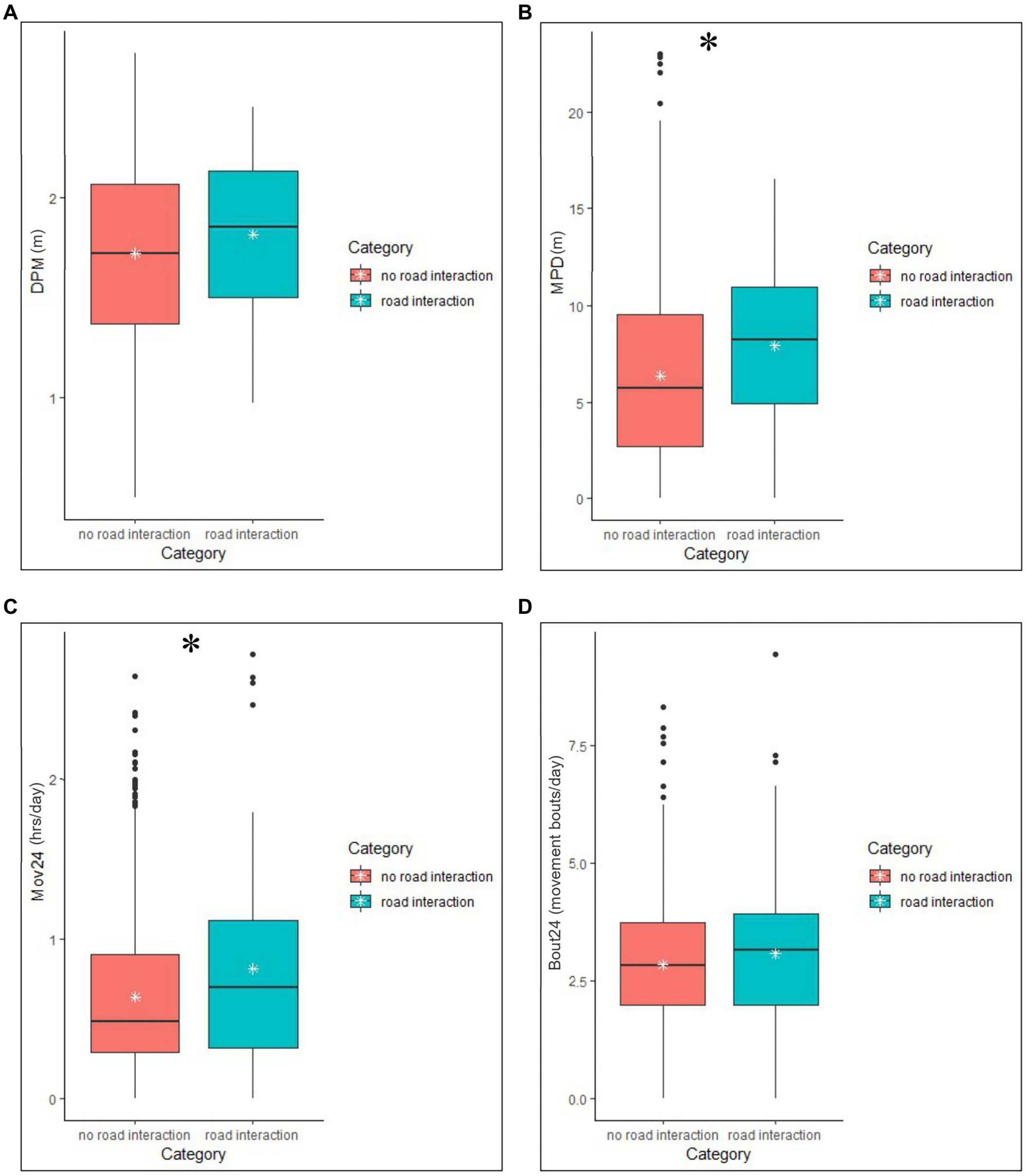

FIGURE 2
Minimum, median, and maximum values and the interquartile range of road interactions and no road interactions in *Crotalus horridus* RT and ACT movement metrics. The white stars on the graphs represent the mean of each category. RT derived movement measures **(A,B)** include Distance Per Movement (DPM) and Meters Per Day (MPD). ACT derived movement measures **(C,D)** include the mean time spent moving per 24h (Mov24) and the mean number of movement bouts per 24h (Bout24). Asterisks denote significant differences ($p<0.05$).

have been documented repeatedly returning to overwintering sites and foraging locations, often across multiple active seasons (Pattishall and Cundall, 2008; Nordberg et al., 2021). In such cases, individuals might travel through disturbed landscapes or cross roads to return to sites used previously, presumably because the benefits of returning to these known sites outweigh the costs of searching for new ones. In our case, two monitored snakes were observed crossing roads while moving to and from their overwintering sites, but neither individual crossed a roadway at any other point during the active season, supporting the possibility that *C. horridus* might endure the risk of crossing roads to return to favorable foraging and overwintering areas used previously. Also, the road surface type, traffic volume, and traffic noise have been found to influence how snakes interact with roads (Robson and Blouin-Demers, 2013). We could not statistically account for these effects in our study, as snakes most often interacted with low traffic, low noise, unpaved roads as opposed to the higher traffic, higher noise, paved roads present at the site. Further sampling could reveal how different road surfaces and traffic volumes might impact *C. horridus* movement behavior in this population and elsewhere.

Intrinsic factors, such as sex and motivational state, have pronounced effects on the movement patterns of pit vipers (Waldron et al., 2006; DeSantis et al., 2019; Emerson et al., 2022) and therefore are a key consideration when investigating the influence of roadways on *C. horridus* movement. For pit vipers, elevated male movement is thought to be the primary determinant of mate location and success, and this is thought to drive significant increases in movement and space

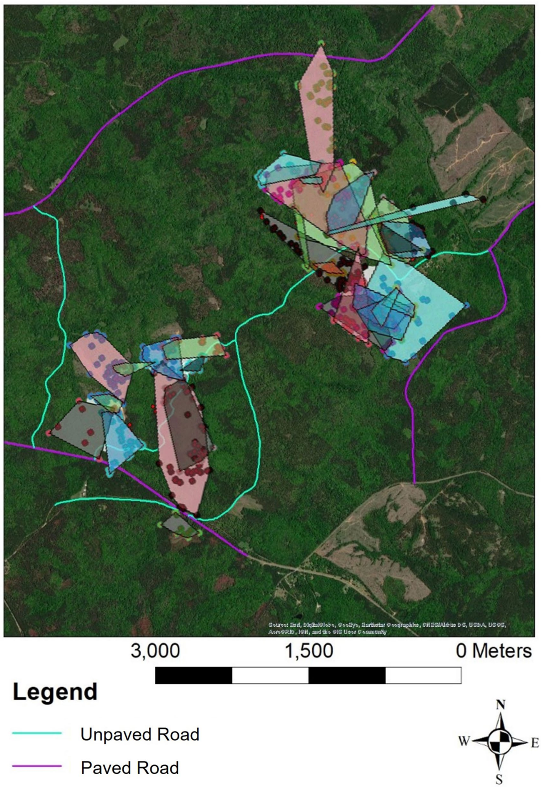

FIGURE 3
Map of the study site within Cedar Creek Wildlife Management Area in Putnam County, GA. Paved roads are outlined in purple, and unpaved, dirt roads are outlined in turquoise. Color coded points indicate the RT locations of each individual snake. 100% Minimum Convex Polygons depict home ranges for all individuals included in the study from 2020 to 2021.

use by males during the mating season (Waldron et al., 2006; Petersen et al., 2019). Given this male-search based mating system and observations made by previous authors on *C. horridus* elsewhere in their distribution, we expected males to generally display elevated measures of movement and space use relative to females (both within seasons and when data are pooled across seasons). As a result of insufficient within-sex and within-season sample sizes, we were unable to statistically evaluate the effect of sex on our annual ACT derived movement metrics or the effect of behavioral season on the annual RT and ACT metrics. However, the annual RT LME models showed that the expected between-sex differences were expressed across several of our RT derived spatial measures of movement and space use. The small effect sizes within our RT LME models that produced statistically significant *p*-values (MPD, DPM, MV and 95% UD) were likely a result of the high variance within our sample when subdividing by sex. Given the abundance of evidence supporting similar sex-specific patterns in other populations of *C. horridus* and congeners (Waldron et al., 2006; Putman et al., 2013; Mata-Silva et al., 2018; Petersen et al., 2019), we are confident that this main effect of sex aligns with general patterns at the population-level for this site. Although males generally had higher measures of movement compared to females, neither were significantly affected by the proximity to roadways. Additionally, the marginal R^2 values for RT and ACT LME models were consistently much lower than the conditional R^2 values indicating that the relationships tested are being heavily influenced by the random effect, snake ID. Clearly, further RT and ACT sampling is needed to better understand the influence of sex and behavioral season on movement in this population. Specifically, it seems especially important to explore whether motivational state (mate-searching vs. foraging) is associated with additional variation in the effect of roads on movement behavior.

Fine-scale road interactions

Although no main effects of DTR were detected for the annual RT and ACT metrics, extracting specific instances when snakes interacted with roads (i.e., when a snake either crossed a road or encountered a road and retreated) allowed for a fine-scale evaluation of variation in movement distances (RT) and durations (ACT). Contrary to our hypothesis, this approach revealed that individuals significantly increased the mean time spent moving per 24 h (Mov24) and the mean distance moved per day (MPD) within the brief periods of time (four relocations, or 2 weeks) containing road interactions. This difference was not reflected in the daily number of movement bouts (Bout24) or the mean distance traveled per movement (DPM). Considering the transient nature and low frequency of these road interactions among our sampled snakes, it is unsurprising that this fine-scale impact of roadways was not maintained at the coarse, annual time scale. Although statistically significant effects of roadways were detected at this resolution, small effect sizes for MPD ($r = 0.14$) and Mov24 ($r = 0.10$) were likely a result of the low frequency of road interaction observations relative to the remainder of the active season, along with high variance in our response variables across those relatively few observations. However, the magnitude of the movement increases observed could still hold biological significance (60.00% increase in Mov24 and 23.14% increase in MPD during road interactions) and highlight the importance of considering the time scale at which movement behavior is evaluated, as relevant variation could be overlooked if the chosen scale is too fine or too coarse. Given that rates of food intake and resulting body condition is a direct predictor of survival and fitness for ambush predators with low energetic requirements (Wasko and Sasa, 2012; Glaudas and Alexander, 2017), the possible energetic costs of increased movement distances and durations during instances when a snake encounters a road could have cascading negative impacts on fitness. Although direct mortality is ultimately the most deleterious fitness consequence associated with frequent road encounters, the sub-lethal consequences of increased movement during road interactions might be amplified in populations where individuals more frequently encounter roads, as chronic increases in time spent moving could lead to excess energy expenditure and a reduction in time spent foraging. When taken along with the increased risks of direct mortality and reductions in genetic diversity for populations exposed to higher road densities and traffic (Clark et al., 2010), this potentially hidden energetic cost from elevated movement during road encounters is an additional factor to consider in snake-road interaction studies. For *C. horridus*, specifically, this also further justifies the recognition of roads as the most prominent threat to populations range-wide (Petersen and Sealy, 2021).

We can only speculate on what might be driving this fine-scale elevation in movement duration and distance among individuals interacting with roads. A possible explanation is that the snakes are displaying an avoidance response by moving back and forth along the road edge prior to attempting to cross. This avoidance behavior could be a response to the road itself or to the open space with little ground or canopy cover created by the road. In previous studies, snakes have been documented to avoid roads (Shine et al., 2004; Andrews et al., 2008; Shepard et al., 2008b; Robson and Blouin-Demers, 2013), and the low frequency of confirmed road interactions during our study period, despite many of the monitored snakes being originally captured on or near roads, supports this hypothesis. Also, there were a low number of road interactions overall, and the snakes that did interact with roads moved away immediately following most road interactions. This does not support the hypothesis that the increased movement of *C. horridus* during road interactions is a result of being attracted to the edge habitat created by roadways (Cowardin et al., 1985; Aresco, 2005; Mata et al., 2017). There is also evidence indicating that snakes are less effective at tracing chemosensory cues across road surfaces relative to the natural landscape (Shine et al., 2004). It is possible that the elevated Mov24 and MPD signal is indicative of indecisiveness when encountering an unusual landscape feature within which they might not be able to effectively perceive the chemical environment. If this is the case, this result would have significant consequences for mate-searching male rattlesnakes in this population and warrants future investigation on the impacts of roads while more effectively accounting for the role of sex and behavioral season. In any case, the fine-scale variation in movement patterns of *C. horridus* revealed in this study emphasizes the value of a scale-dependent analytical approach and demonstrates the utility of the recently validated RT-ACT framework in fine-scale behavioral monitoring of pit vipers.

Conclusion

Negative effects of roads on vertebrate taxa have been widely documented (Ashley and Robinson, 1996; Shepard et al., 2008b; Clark et al., 2010), but the behavioral mechanisms mediating wildlife-road interactions have received far less attention, especially for small and secretive species such as most snakes. This study is among the first systematic uses of accelerometry for quantifying the movement behavior of snakes in the field, and the first to leverage RT-ACT monitoring for detailed evaluation of the scale-dependent behavioral responses of snakes to roadways. Annual, coarse scale RT and ACT measures of movement and space-use did not reveal differences in spatial movement patterns or daily movement durations in relation to mean distance from roads, likely as a result of the large and unfragmented patches of habitat adjacent to roads at our study site. However, quantifying RT and ACT movement metrics at a much finer time scale uncovered a significant increase in movement distances and durations by *C. horridus* during the rare instances when they did encounter roads. This finding has appreciable conservation implications for all *C. horridus* populations. In addition to the widely referenced negative effects of road mortality on *C. horridus* and many other snake species (Andrews et al., 2008; Clark et al., 2010; Petersen and Sealy, 2021), this potential hidden energetic cost incurred during road encounters could have significant fitness effects, especially for those populations occurring in habitats more highly fragmented by roads. While we were unable to consider the effects of road surface type and traffic volume in this response due to limited sample sizes, it is notable that 15 of the 17 road interactions included in the fine-scale analysis involved low-traffic, unpaved roads, perhaps indicating that *C. horridus* were more likely to interact with low-use unpaved roads than the relatively higher-trafficked paved roads in the study area. This highlights the importance of considering the effects of low-traffic roads on snake behavior even in the absence of prominent road mortality. Ultimately, further sampling is required to refine our understanding of the effects of different types of roads on the movement behavior of *C. horridus*, specifically to better account for the roles of sex, season, and motivational state. Nevertheless, our results highlight the value of this novel integration of radio telemetry and accelerometry for quantifying snake movement behavior across multiple time scales, particularly in attempts to capture subtle or fleeting instances of behavior with potentially important fitness consequences.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Georgia College & State University (protocol number 2020-D). The work was permitted by the Georgia Department of Natural Resources (Scientific Collecting Permit #1001056844).

Author contributions

The project was designed by AT and DD. AT contributed to data collection and analyses and led manuscript preparation. DD contributed to data collection and analyses and critically revised the manuscript. JV-D contributed to the accelerometer data processing, model validation, and application, and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research was provided by the GCSU Department of Biological and Environmental Sciences, GCSU College of Science, and the Herpetologists' League E. E. Williams Research Grant.

Acknowledgments

We would like to thank Liz Caldwell, Daniel Sollenberger, Zach Nichols, Cliff Rushton, David Lutz, and Paul Watson for providing study site access and logistical support for this research. We would also like to thank Reagan Thornton, Conor Evans, and Morgan Thompson for their assistance with field data collection. Finally, we thank Christine Mutiti for assistance with spatial data processing and Katie Stumpf and Matthew Milnes for their helpful feedback on early versions of this manuscript. Content in this manuscript appeared previously online in author AT's thesis (Tipton, 2022).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 16 February 2023
DOI 10.3389/fevo.2023.1074072

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SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 19 October 2022
ACCEPTED 18 January 2023
PUBLISHED 16 February 2023

CITATION
Conan A, Le Brishoual M, Garnier L, Fleitz J, Dehaut N, Enstipp M, Jumeau J and Handrich Y (2023) Efficacy of permanent wildlife fences as barriers to amphibian movement.
Front. Ecol. Evol. 11:1074072.
doi: 10.3389/fevo.2023.1074072

Efficacy of permanent wildlife fences as barriers to amphibian movement

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To mitigate habitat fragmentation and roadkill, roads are increasingly equipped with wildlife fences and underpasses. However, the effectiveness of such fences in preventing road access for amphibians has not been tested under controlled conditions. In 2019 and 2020, we tested the efficacy of full panel fences of differing material, height, and shape (presence/absence of an overhang), to prevent road access for adult and juvenile amphibians. We selected five species according to locomotion mode: Natterjack toads (*runners*), European green toads (*short-distance jumpers*), agile frogs (*proficient jumpers*), American tree frogs (*proficient climbers*) and smooth newts (*climbers*). We found that Natterjack and green toads were unable to cross a concrete fence with a height of 13 and 24 cm, respectively. Addition of a 10 cm overhang reduced the height required to prevent crossing further to 10 and 17 cm, respectively. The ability of these less agile species to cross a certain fence height depended on body length. By contrast, jumping agile frogs and climbing tree frogs were not stopped by the greatest fence height tested (40 cm). However, addition of the overhang stopped the climbing tree frogs at a concrete fence height of 35 cm. An alternative metal fence (with overhang) was tested with some species and performed similar to the concrete fence (with overhang). Finally, the greatest concrete fence height passed by climbing juveniles was 20 cm (smooth newts). Hence, to stop amphibians from road crossing, we recommend the construction of durable (concrete or galvanized metal) and well-maintained fences with a minimum height of 40 cm with a 10 cm overhang.

KEYWORDS

movement barriers, mitigation, road ecology, amphibians, conservation

1. Introduction

Linear transport infrastructures, such as roads, are a threat to biodiversity and one of the most significant causes of landscape fragmentation (Maxwell et al., 2016). These structures lead to habitat loss, roadkill, and disrupt the movement of animals by creating ecological barriers (Fahrig et al., 1995; Forman and Alexander, 1998). While most taxa are impacted by roads (Forman and Alexander, 1998), some are particularly sensitive to them. According to the European Red List of Amphibians (Temple and Cox, 2009), habitat loss and degradation are the most significant threat to amphibians, affecting 76 of the 85 species found in Europe (Stuart et al., 2004; Maxwell et al., 2016). Many amphibians undertake spring and autumn migrations, which renders them particularly sensitive to roads between the various key areas used during the different phases of their life cycle (Wilbur, 1980; Miaud et al., 2000; Semlitsch, 2008; Joly, 2019; Cayuela et al., 2020). Even outside the migration periods, roads disrupt the movement of individuals between local populations and, furthermore, increase mortality through roadkill, potentially leading to the extinction of local and regional amphibian populations (Fahrig et al., 1995; Dodd et al., 2004; Petrovan and Schmidt, 2016; Testud and Miaud, 2018; Joly, 2019).

To limit roadkill and restore connectivity, mitigation measures have been implemented. These measures are numerous and include, for example, traffic and speed reduction, road closures and wildlife passages (Schmidt et al., 2008; Rytwinski et al., 2016; Testud and Miaud, 2018). The last measure is often associated with fences (e.g., wire netting, plastic mesh, or full panel concrete or metal), constructed to prevent animals from venturing onto roads and guide them instead towards passages (under/overpasses), where they can safely cross a road (Schmidt et al., 2008; Arntzen et al., 2017; Carvalho et al., 2017; Testud and Miaud, 2018). These fences are typically designed for large fauna (large mesh-size), such as ungulates (e.g., deer; Fahrig et al., 1995; Romin and Bissonette, 1996; Clevenger and Waltho, 2000; Forman et al., 2003; Dodd et al., 2004; Glista et al., 2009), but often contain additional components (fences with a small mesh-size), to stop amphibians and small mammals (Morand and Carsignol, 2019). Large fauna fences have been relatively well studied (i) because they are of obvious interest for human safety (Romin and Bissonette, 1996; Schwabe et al., 2002; Forman et al., 2003; Bouffard et al., 2012) and (ii) because their effectiveness can be easily demonstrated by monitoring the number of collisions between ungulates and vehicles.

For small fauna, different types of fences have been used but their effectiveness has rarely been studied. The few studies that have been carried out suggest a low efficacy of such fences (Dodd et al., 2004; Woltz et al., 2008; Brehme et al., 2021; Conan et al., 2022). For example, Arntzen et al. (1995) tested the effectiveness of chicken wire (40 cm height, 10 cm overhang, 1.3 cm mesh size) and plastic mesh fences (50 cm height, 10 cm overhang, 0.3 cm mesh size) to avoid amphibian road crossings (toads and newts, respectively). They found that chicken wire reduced the number of crossings by between 35 and 70% for toads, while plastic fences reduced the crossings of newts by between 34 and 48%. The findings of the above and further studies indicate that plastic mesh fences allow a relatively high number of amphibians to pass and, in some cases, do not reduce roadkill (Arntzen et al., 1995; Baxter-Gilbert et al., 2015; Ottburg and van der Grift, 2019). Plastic fence heights tested in these studies ranged from 40 cm for amphibians to 80 cm in reptiles and no overhang was used. Another type of small wildlife fence regularly found along roads is a wire netting fence (with adapted mesh size). However, this type suffers from the same defects as the plastic mesh fence; it deteriorates quickly and is easily climbed and passed by small-mammals and amphibians (Conan et al., 2022). Non-mesh (full panel) fences, constructed with different materials (e.g., PVC, concrete and metal) and a typical height of 40–60 cm (Morand and Carsignol, 2019), have also been used alongside roads. Such fences are fairly durable, overcoming the problem of fast deterioration and are, therefore, increasingly recommended (Dodd et al., 2004; Morand and Carsignol, 2019; Conan et al., 2022). However, to the best of our knowledge, only one study tested their efficacy for amphibians under controlled conditions in the laboratory (Zbierski and Schneeweiß, 2003). That study, which tested the efficacy of different shapes of full panel concrete fences (40–60 cm height without overhang) for European tree frogs (*Hyla arborea*), found that the most effective shapes are difficult to build and expensive to maintain (Zbierski and Schneeweiß, 2003).

1.1. Research objectives

In the current study, we investigated the efficacy of different types of full panel fences in preventing road access with five European amphibian species. We tested fences (1) made from two types of materials (concrete or galvanized metal) (2) of different height, and (3) without or with a horizontal metal overhang. To achieve more generalizable results, we selected five species with different locomotion modes (i.e., runner/jumper/climber) and included different developmental stages (adult and juvenile). We further tested fences in a dry or wet state (simulating the most common meteorological conditions) and also considered individual morphological measurements in our analysis.

We expected that: (1) A fence height exists that prohibits the crossing for all amphibian species tested (effective fence height; H_{eff}) and this height will differ between fence types; (2) given the differences in locomotion mode of the species/developmental stages tested, H_{eff} will differ between species/developmental stages; (3) the presence of an overhang will improve the efficacy of fences tested; (4) wetting of the fence will increase the crossing capacity of climbers; (5) the larger size and/or better body condition of some individuals will allow them to pass fences at a greater height than others.

2. Materials and methods

2.1. Model species, capture conditions and temporary captivity

Five amphibian species with different modes of locomotion in the adult stage were used in our study (Table 1). The Natterjack toad (*Epidalea calamita* Laurenti, 1768) moves by running on the ground; the European green toad (*Bufotes viridis* Laurenti, 1768) advances by small

TABLE 1 Information on studied species and capture dates.

Species	Stage	N	Mode of locomotion	Origin	Capture date
Natterjack toad (*Epidalea calamita*)	adult	12	running	Field	July 2019
	juvenile	20	climbing	Field	July 2019
European green toad (*Bufotes viridis*)	adult	12	jumping (short-distance)	Field	June 2020
	juvenile	20	climbing	Field	July 2020
American tree frog (*Dryophytes cinereus*)	adult	8	climbing (proficient)	Pet shop	September 2020
Agile frog (*Rana dalmatina*)	adult	15	jumping (long-distance)	Field	March 2020
	juvenile	20	climbing	Field	June 2019
Smooth newt (*Lissotriton vulgaris*)	juvenile	20	climbing	Field	July 2019

Only males were used at the adult stage; sex was not determined for juveniles.

steps (low height/length ratio); the American tree frog (*Dryophytes cinereus* Schneider, 1799) is a good climber, due to its adhesive toepads (good adhesion on smooth surfaces, Li et al., 2021), and the agile frog (*Rana dalmatina* Fitzinger in Bonaparte, 1839) makes powerful jumps. The smooth/common newt (*Lissotriton vulgaris* Linnaeus, 1758) walks on the ground but can also climb walls by adhesion to the substrate. Given the low body mass and small size of juveniles, we assumed that juveniles of all species tested would be able to climb walls by adhesion. Hence, the behavior when crossing a vertical obstacle might differ between the developmental stages of species tested. During preliminary tests with adult male and female Natterjack toads, some females started to oviposit outside the water and without the presence of males. To limit any potential impact of our experimentation on wild populations, we consequently excluded adult females from experimentation and only included males at the adult stage. Any potential bias in our results, due to testing males only, should be limited to Natterjack toads, European green toads and tree frogs, for which size differences between sexes are small (Leskovar et al., 2006; Oromi et al., 2012). Female agile frogs are typically larger than males (~14%; Angelini et al., 1995), so that we cannot exclude a potential bias in our results for this species. However, our results suggest that this is unlikely an issue (see below).

Due to the national lockdown that occurred during the first Covid-19 outbreak in France (March–May 2020), we were unable to capture wild European tree frogs (*Hyla arborea* Linnaeus, 1758). Given their morphological similarity, we used American tree frogs *(Dryophytes cinereus)* as a substitute species. The lockdown also prevented us from completing some trials with Natterjack toads and agile frogs, so that only two fence types were tested with these species (concrete without or with a 10 cm overhang).

With the exception of American tree frogs that were purchased from a pet store ('La ferme tropicale', Paris, France), all individuals of the other species (both stages) were captured near their breeding sites during their terrestrial phase (see Table 1 for the number of individuals and capture dates). To minimize any potential effect on local populations, we captured individuals only at breeding sites with a sufficiently large population and which were close to the place of experimentation. Individuals were collected by hand (with gloves) or with a net during nocturnal patrols. After experimentation, individuals of all species except American tree frogs were released at the site of capture, following a maximum of 35 days in captivity. Individuals of the latter species could not be released into the wild and remained in captivity as pets.

To allow individual tracking during experimentation, a RFID PIT tag (1.4 × 9 mm; TAG LF GLT1M4X9 RO EM, Biolog-ID®, Bernay, France) was implanted into adults at the time of capture. Following implantation, the following morphological measurements were taken from adults: (1) body mass in g (BM), (2) snout-vent length in mm (SVL, from the snout to the tip of the ischium) and (3) extension length in mm (EL, the length of the straight line between the tips of hindlimbs and forelimbs, with both limbs extended, i.e., elongation size). Body mass was determined with a spring balance (0–50 g, resolution: 0.5 g, accuracy: ±0.3 g; Pesola®, Schindellegi, Switzerland), while SVL was measured using an electronic caliper (0–150 mm, accuracy: ±0.03 mm, Tesa technology®, Renens, Switzerland). The precise measure of EL was difficult with active amphibians; we used a tape measure (Dexter, Lezennes, France) and rounded measurements to the nearest 5 mm. A body condition index (BCI) was calculated for all species following Green (2001), using the individual residuals of the linear regressions between Ln(BM) and Ln(SVL).

Throughout captivity, individuals were kept in 800 × 600 × 435 mm polypropylene tanks with perforated lids (Euronorm container 12.4046.07 AXESS industries®, Strasbourg, France). Individuals were housed together according to species and developmental stage. The maximum number of individuals per tank was 20 (i.e., juveniles). The tanks were enriched with moist, sandy soil and fresh moss. Flat tiles and stones provided hiding places for individuals. Finally, during experimentation (which coincided with the breeding season), food was available *ad libitum* in the tanks [e.g., earthworms (*Lumbricidae*, ~5 cm), wax moth (*Galleria mellonella*, ~2 cm) and crickets (*Acheta domesticus*, ~1 cm)].

All procedures were carried out after obtaining the authorizations for capture and transport (2019-DREAL-EBP-0031) and a certificate for the detention of the various species in captivity (DDPP67-SPAE-FSC-2019-04). The experimental protocol was approved by the French Ethical Committee (CREMEAS) and the French Ministry of Environment: 'Ministère de la Transition Écologique' under agreement number (APAFIS #18546-2019011810282677.v7).

2.2. Experimental setup

2.2.1. Test arena

All tests were conducted in an arena built from PVC (60 × 80 × 70 cm, width*length*height; Figures 1, 2) that was placed inside a large semi-natural outdoor enclosure (2000 m^2 large fenced in area that excluded predators). The arena consisted of three compartments: (1) the 'departure' compartment (~60 × 30 cm) had a bare concrete floor (i.e., unattractive), while the (2) 'arrival' compartment (~60 × 40 cm) was enriched with wet soil and moss, hides made of bricks and a small pool filled with freshwater. The latter was added as an attractive reward and has been used to that end in learning experiments with toads (Dall'antonia and Sinsch, 2001; Daneri et al., 2007). On the rear side of the 'arrival' compartment a speaker (Pulsar® 2 × 3 W speaker, Enkhuizen, Netherlands) replayed calls from *Epidalea calamita* and *Pelophylax* spp., to motivate amphibians to cross the fence. Male calls (conspecific or not) are known to attract all resident amphibian species (Gerhardt, 1995; Yeager et al., 2014; Muller and Schwarzkopf, 2017). Adjacent to the 'arrival' compartment was (3) a further compartment (~60 × 10 cm) that hosted two males of the tested species, which added to the acoustic stimulus provided by the speaker, while also potentially relaying visual and olfactory cues. The first two compartments were separated by the fauna fence to be tested (concrete or galvanized metal fence), while the last compartment was separated from the arrival compartment by a perforated Plexiglass plate (Figure 1). The top of the arena was covered with a regularly perforated Plexiglass lid to allow for good ventilation and its openings were covered with netting. An air ventilation system (Pump KNF N840.1.2FT.18, Freiburg im Breisgau, Germany) provided appropriate ventilation throughout the arena and also allowed for an exchange of scent throughout the system (i.e., amphibians in the departure compartment likely smelled their conspecifics in the last compartment). To test the effect that moisture might have on the crossing capacity of amphibians, half of the length of the fence tested (concrete and galvanized metal) was fitted with a water circulation system (aquarium pump; Neptus Mini P300®, Italy) that permanently wetted this part of the fence (Figure 2).

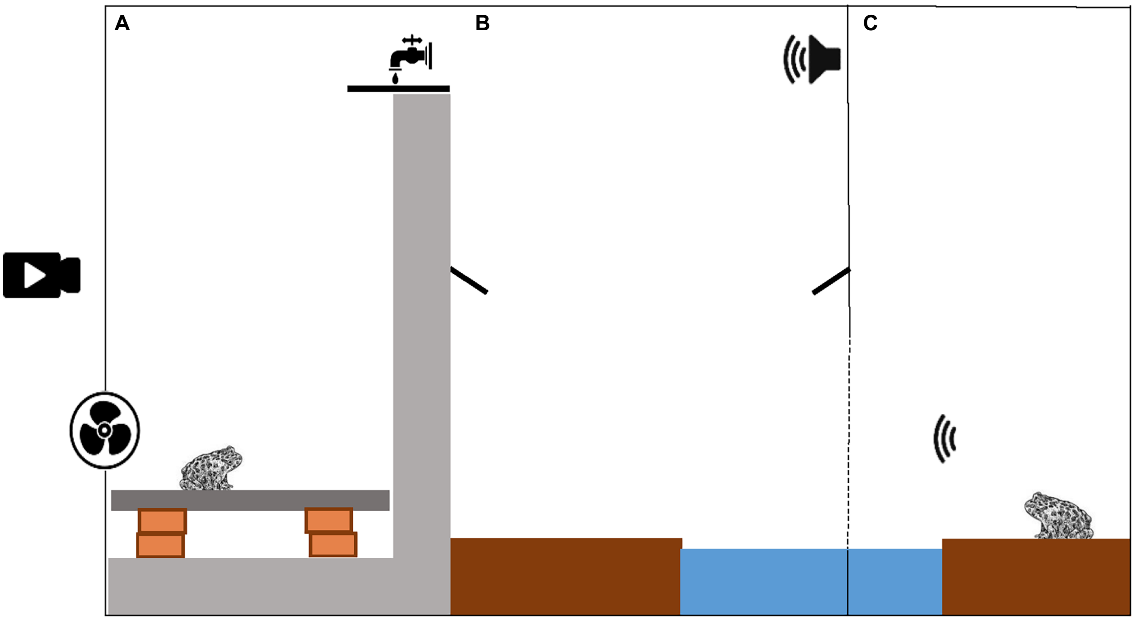

FIGURE 1
Illustration of the experimental arena. The aquatic part is shown in blue, the enriched soil in brown, the fauna fence is shown in grey and the overhangs are represented by solid black lines. The iconography from left to right: camera; ventilation system; species tested; water inlet to wet fence; loudspeaker; singing male. **(A)** 'Departure' compartment (not enriched) with a movable concrete base (shown in dark grey) that allowed modification of test height (see also Figure 3); **(B)** 'arrival' compartment, enriched with sandy soil and moss; **(C)** compartment with singing males, separated from the 'arrival' compartment by a perforated Plexiglass plate (covered with a net), allowing the exchange of visual and olfactory cues in addition to auditory stimulation. Overhangs in the 'arrival' compartment were added to prevent individuals that crossed the fence to return to the 'departure' compartment.

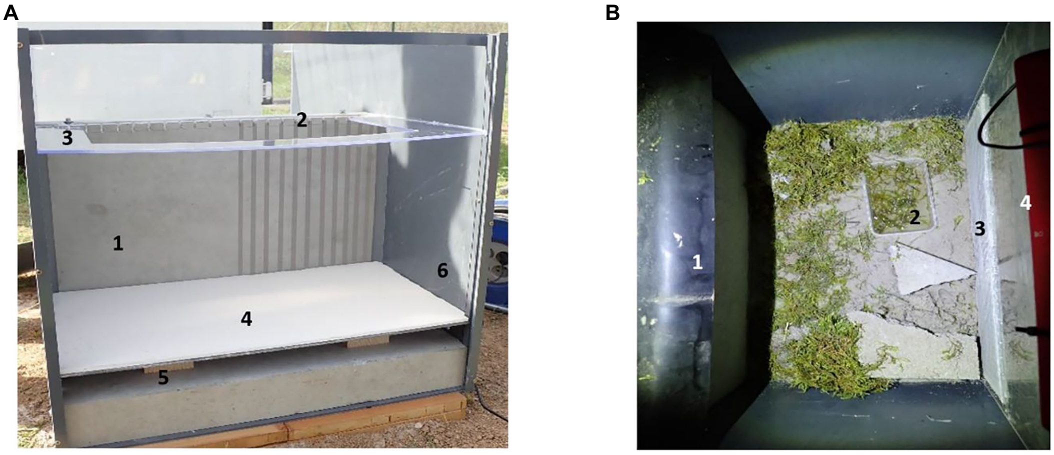

FIGURE 2
Experimental arena. **(A)** Frontal view of the 'departure' compartment: (1) dry part of the vertical concrete fence tested; (2) wet part of the vertical fence tested; (3) anti-escape Plexiglass plate; (4) movable concrete base (30x50 cm, width*length), allowing to adjust fence height; (5) wooden blocks for height adjustments; (6) transparent frontal Plexiglass plate allowing video recording; **(B)** arial view of the 'arrival' compartment: (1) Overhang made from a 7.5 cm wide PVC sheet, that was fixed 5 cm below the top of the fence and bent downwards (inclination angle: ~75°), to prevent animals from returning to the 'departure' compartment; (2) small water tank placed among other enrichments: sand, wet moss, hides made of bricks; (3) opening in Plexiglass plate (covered with a mesh) enabling ventilation throughout all compartments; (4) speaker that replayed calls of *Epidalea calamita* and *Pelophylax* spp., to stimulate amphibians to cross the fence.

2.2.2. Tested obstacles

Three full panel fence types were tested: (1) a concrete fence without overhang (Crt), (2) a concrete fence with a 10 cm horizontal overhang (Crt+) and, lastly, (3) a fence of galvanized metal (Galv) with a bent overhang that extended 5 cm horizontally and 2 cm downwards (Figure 3). The overhang dimensions for the concrete fence were chosen according to results of a previous study, in which a 10 cm overhang was sufficient to prevent the crossing of green toads and marsh frogs (*Pelophylax ridibundus*; Conan et al., 2022). The bent overhang for the metal fence was required to provide structural stability and the dimensions used were readily available commercially. To allow testing of different fence heights, the movable base of the departure

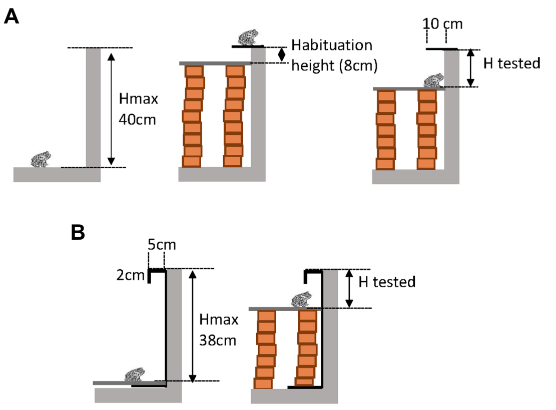

FIGURE 3
Setup to test the capacity of amphibians to cross different fence types and heights. We used wooden blocks to adjust the concrete base to the desired fence height to be tested. All tests with adult amphibians started with a habituation height of 8 cm, before height was increased in the following trials. **(A)** Concrete fence tests: Hmax (the maximal height tested) was 40 cm, without or with an overhang of 10 cm; **(B)** galvanized metal fence tests: Hmax was 38 cm; this fence type had a bent overhang (5 cm horizontal +2 cm downwards).

compartment was mounted onto a varying number of wooden blocks, altering the distance to the top of the fence (Figure 3). As stated above, due to the Covid crisis lockdown, we were unable to test all fence types with all species/developmental stages.

2.3. Test protocol

2.3.1. Experimental trials

The primary goal of our trials was to find the lowest effective fence height (H_{eff}) for all fence types tested (Crt, Crt+, Galv) that prevented crossing for individuals of all species and developmental stage.

During experimental trials, individuals were tested in groups of 8, 10 or 20 individuals of the same species and developmental stage (Table 1). The difference in numbers resulted from permit limitations for adults (N = 10) and the inability to purchase more than 8 tree frogs, while we were able to include a greater number of juveniles (N = 20) per species. At the start of a trial, animals were placed into the 'departure' compartment at the end of a day (9:30 PM) and had until the following morning (7:30 AM) to cross the fence. During the entire trial duration, calls were replayed from the speaker (looped recording), callers were present inside the last compartment and a video camera recorded continuously (Figure 1). The following morning, after completion of a trial, individuals that had managed to cross the fence were removed from the 'arrival' compartment, counted and identified (RFID tags in adults only). Thereafter, all individuals that participated in a trial were transferred back to their holding tanks. To allow animals to familiarize themselves with the setup, a fence height of 8 cm (i.e., habituation height, Figure 3) was used during the first night for each group (except juveniles, see below). Following that first night, animals were tested for a maximum of three consecutive nights for a particular height. We started with the maximum height possible for the different fence types (40 cm for the concrete fence types and 38 cm for the galvanized metal fence) and iteratively decreased or re-increased height in the following trials, depending if individuals failed or succeeded to cross. As soon as at least one individual managed to cross a particular height during one night, height was increased during the following night. For example, if amphibians failed to cross the 40 cm fence for 3 consecutive nights, the following night, fence height was adjusted to half the difference between the habituation height (8 cm) and the maximal height (40 cm), hence, to 16 cm (32/2 cm). If one individual managed to cross at that height, fence height was increased to 24 cm (16 + 8 cm). Testing continued until H_{eff} was found for a given species, stage, and type of fence.

Apart from slight modifications indicated below, testing conditions for juveniles of all four species were identical to adults. Juveniles were tested in groups of 20 individuals and with a strict limitation on the duration of experimentation. Given the fragility of amphibians during their juvenile stage, we limited experimentation to 3 consecutive nights and did not offer a habituation trial, as in adults. The first night, the height to be crossed was set to 40 cm with a 10 cm overhang (maximum difficulty possible to test with our setup), which was never reached by climbing juveniles. To estimate the greatest height reached by juveniles, we attached a measuring bar to the concrete fence, that was clearly visible in the video footage. During the following 2 nights, fence height was set to half the height reached by climbing juveniles during the previous night (as identified from video footage), to encourage successful crossing. Unfortunately, no further height increments could be tested because of the set time limit for experimentation with juveniles. This also prevented us from testing the concrete fence with overhang and the metal fence with juveniles.

Following a trial, we viewed the recorded video footage to identify how individuals crossed the fence. Our protocol was designed to strongly motivate individuals to cross the fence. Besides call replays, the presence of conspecifics (callers) in the last compartment and the enrichment inside the arrival compartment, trial durations were long (10 h) and each height was tested during a maximum of 3 consecutive nights (if amphibians failed to cross). Hence, we presume that a fence height not crossed during our experimentation will not be crossed in the wild.

2.4. Analysis and statistics

2.4.1. Test of protocol efficacy

To ensure that our protocol sufficiently motivated individuals to cross the fence and to further quantify the number of crossing attempts of individuals, we conducted a behavioral analysis on 10 h of video footage from European green toads and Natterjack toads. We chose footage from unsuccessful trials (i.e., no individuals passed the fence) and studied how the number of crossing attempts varied between individuals. Since adult amphibians were implanted with an RFID tag, we could investigate individual differences in crossing success and also determine if crossing failures were likely explained by height or differences in motivation (based on the number of observed attempts). For example, it is possible that individuals pass at a greater height but fail to do so at a lower height, simply because they were not motivated to cross.

2.4.2. Effective fence height (H_{eff}) and the effects of morphology

Our goal was to experimentally determine H_{eff} for all conditions and species/developmental stages. We found clear differences in H_{eff}

between species/developmental stages depending on locomotion mode and their strategy to cross the fence. However, since we could not determine H_{eff} for all species, statistical testing was restricted to European green toads and Natterjack toads, the least agile species tested. We used an ANOVA to test for differences in H_{eff} between the latter two species and linear regression analysis to investigate the relationship between their morphological features (EL, SVL, and BM) and H_{eff}.

2.4.3. Effects of fence wetting

To investigate whether wetting of the fence would increase the crossing capacity of climbers (i.e., tree frogs and all juveniles), we used the recorded video footage to determine the number of crossing attempts during 12 randomly selected 15 min periods, and recorded whether crossing attempts occurred on the dry or wet side of the fence. Unfortunately, the video footage for all juveniles, except Natterjack toads, did not allow a systematic investigation (condensation fogging up the image). Hence, this analysis was conducted for juvenile Natterjack toads and adult tree frogs only. We used a generalized linear model (GLM) with a quasi-poisson distribution to test for differences in the number of crossing attempts between the wet and dry part of the fence for both species.

Analysis and graphs were conducted with R (v3.5.3, RStudio v1.2.1335 and GraphPad Prism 8.2.1). All results are shown as mean ± SEM with a statistical significance threshold of 5%.

3. Results

3.1. Test of protocol efficacy

During the 10 h of video recording we analyzed, all individuals engaged in crossing attempts. We recorded 735 and 344 unsuccessful crossing attempts of European green toads and Natterjack toads, respectively. On average, individual green toads made 8.4 ± 0.1 attempts per hour (range 4–13), while Natterjack toads engaged in 3.9 ± 0.1 attempts per hour (range 3–11). The frequency of crossing attempts remained stable throughout the night in both species. Considering all tests conducted, 50% of European green toads, failed to cross at a lower height but managed to cross at a greater height later on, while this did not occur in Natterjack toads. In addition to the video analysis conducted with the two toad species, analysis of the RFID tag data showed that, similar to the green toads, agile frogs (80%) and American tree frogs (75%) passed at a greater height after having failed to cross at a lower height.

3.2. Efficacy of fence types in preventing amphibian road access

The effective fence height (H_{eff}) for all species and developmental stages tested with different fence types is shown in Table 2. Only the nimblest species tested (agile frogs and American tree frogs) were able to cross a full panel concrete fence (without overhang) at a height of 40 cm (the maximum height we could test). Furthermore, addition of a 10 cm overhang decreased the crossing success for all species, except for agile frogs (Crt vs. Crt+; Figure 4; Table 2). However, given the height limitations, we were unable to determine the effective fence height for the concrete fence without overhang for agile and tree frogs. In the following, results are presented separately for the nimblest species (agile and tree frogs), the toad species (green and Natterjack toads), and juveniles of all species.

3.2.1. Adult agile frogs (proficient jumpers) and American tree frogs (proficient climbers)

With a single direct jump, agile frogs were able to reach the top of the concrete fence. However, they required a sufficient minimum distance to the fence (~30 cm), especially when jumping in a straight fashion towards it. Successful attempts at maximum fence height were mostly sideway jumps, when individuals took advantage of the 60 cm width of the compartment (see Figure 2) to increase distance to the fence, so that they could reach a sufficient height during the jump. The presence of an overhang, which reduced the distance by 10 cm, did not prevent any individuals from reaching the top of the 40 cm Crt+ fence. Fence crossing in this species often involved two steps: firstly, an individual would jump to the ‘Anti escape Plexiglas sheet’ (see #3 in Figure 2), located at a height of 40 cm, and then jump further to the top of the fence or even directly into the ‘arrival’ compartment. The greater distance possible when jumping first to the Plexiglass plate possibly contributed to the ease with which agile frogs reached the fence top. However, such a strategy is likely irrelevant in the context of wildlife fences, when such a structure will not be present. Unfortunately, the galvanized fence type could not be tested with agile frogs.

Similar to agile frogs, American tree frogs passed over the maximum fence height tested (40 cm concrete fence, without overhang) during the first night of testing (Figure 4). However, they used a different mode of locomotion; rather than jumping, they climbed the concrete fence and showed a significant preference for the wet part of the fence ($t = 2.1$, $p = 0.04$; Figure 5). In addition, tree frogs developed a different mode to cross the fence after ~8 nights. Individuals would climb the smooth PVC sidewalls or the smooth frontal Plexiglass plate and then jump onto the fence top or right into the arrival compartment. Hence, unfortunately, when testing the concrete fence with overhang (Crt+), tree frogs did not try to cross the 40 cm fence directly but used their newly developed mode instead. Nonetheless, one individual passed the overhang *via* the direct fence route at a height of 35 cm. Testing the galvanized fence (Galv) with the tree frogs showed that the 2 cm downward component of the overhang (see Figure 3B) was rather counterproductive. Frogs were able to cling to it and, by stretching out, they managed to reach the fence top and cross over the fence. Nevertheless, tree frogs were unable to cross the maximal metal fence height of 38 cm directly. Similar to the Crt+ tests, frogs only managed to cross at that height by climbing the side walls and then jumping over the metal fence.

3.2.2. Adult Natterjack toads (runners) and European green toads (short distance jumpers)

The only way adult Natterjack toads managed to cross the tested fences was by stretching out and pulling themselves up and over the fence top. By contrast, adult green toads often jumped onto the fence and then stretched themselves towards the top to cross over. Given the jumping advantage of green toads, they managed to cross all fence types at a greater height than Natterjack toads ($F = 118$; $d = 1$; $p < 0.001$; Figure 4; Table 2).

3.2.3. Juvenile amphibians

The only mode by which juveniles attempted to cross the concrete fence without overhang (the only type tested with juveniles)

TABLE 2 Summary of experimental trials according to species and development stage.

Groups tested		Test duration (Nr. of consecutive nights)				Smallest effective fence height: H_{eff} (cm)		
Species	Stage	Test 1=concrete without overhang	Test 2=concrete+ overhang	Test 3=galvanized fence (with overhang)	Total	Concrete without overhang ('Crt')	Concrete with overhang ('Crt+')	Galvanized fence ('Galv')+ overhang
Natterjack toad	adult	9	4	Nt	13	13	10	Nt
European green toad	adult	20	7	8	35	24	17	19
American tree frog	adult	5	10	5	20	> 40	35	36
Agile frog	adult	2	2	Nt	4	> 40	> 40	Nt
Smooth newt	juvenile	3	Nt	Nt	3	21	Nt	Nt
Natterjack toad	juvenile	3	Nt	Nt	3	12	Nt	Nt
European green toad	juvenile	3	Nt	Nt	3	ND	Nt	Nt
Agile frog	juvenile	3	Nt	Nt	3	16	Nt	Nt

Nt = not tested; ND = not determined.

was by climbing. Juvenile European green toads were the exception and never attempted to cross the fence. However, the juveniles of all other species were able to climb the fence by adherence of their extremities. Similar to adult tree frogs, juvenile Natterjack toads preferentially used the wet side of the fence ($t = 2.1$, $p = 0.04$; Figure 5). This was likely also the case for the juveniles of the other species but could, unfortunately, not be determined in our analysis. With the exception of the green toads, all juveniles were able to climb to a height equivalent to more than 10 times their size (SVL ~1 cm; Figure 4; Table 2). However, no juveniles were able to cross a fence height greater than 20 cm.

3.3. Effects of morphological parameters on minimum effective fence height (H_{eff})

Morphological features differed between species (Table 3). While green toads were the heaviest, agile frogs were the longest of all species measured. Regression analysis showed significant relationships between all morphological variables measured and H_{eff} for Natterjack toads (Table 3; Figure 6). By contrast, this was not the case for green toads (Table 3; Figure 6). Hence, larger Natterjack toads were able to cross at a greater fence height than their smaller conspecifics (Figure 6). This is likely also true for green toads but given our small sample size, relationships failed to reach significance.

4. Discussion

Our experiments showed that the height of the fence types tested was sufficient to stop adult individuals of species representing the *running* and *short-distance jumping* mode (Natterjack and European green toads), as well as all juveniles from crossing. Addition of an overhang (in case of the concrete fence) further reduced height at which these species were able to cross the fence. By contrast, proficient jumping and climbing species (American tree frogs and agile frogs) were not stopped by the greatest fence height tested. However, addition of an overhang stopped the proficient climbers (tree frogs) at a height below the maximal tested height, while it had no effect on agile frogs. An alternative galvanized metal fence (with overhang), which we tested with some species, performed similar to the concrete fence with overhang. Finally, climbing juveniles were only able to pass a concrete fence at about half its maximal height and only when it was wet.

In our setup, amphibians were highly motivated to cross the barrier separating the departure and arrival compartment. When failing repeatedly to cross at a particular height, individuals nevertheless continued to try. Crossing attempts in such cases ranged between 3 and 13 per hour for the two toad species tested. Furthermore, if amphibians failed to cross at a particular height, they were given two more nights (3 consecutive nights per test height in total). A sufficient time frame for testing is important, since motivation to cross might not be identical throughout trials. For example, we observed that individuals of all adult species, except Natterjack toads, failed to cross the fence at a lower height but managed at a greater height soon after. Accordingly, results from a previous study that are based on only 6 h of testing and without replicates should be treated with caution, as they might underestimate the crossing capacity of species (Zbierski and Schneeweiß, 2003). In the wild, the motivation to cross might differ with season, breeding status, rain and further parameters. Our experimental setup consisted of a small test arena and we used a number of stimuli to ensure sufficient motivation for crossing. Hence, we believe that our setup was well suited to test the crossing capacity of amphibians.

As expected, we found that the effective fence height (H_{eff}), i.e., the minimal height at which amphibians failed to cross, differed according to locomotion mode. However, in contrast to our most fundamental expectation, a concrete fence without overhang (Crt; maximal height tested: 40 cm) was only sufficient to stop the adults of the less nimble species, like toads (green toads and Natterjack toads) and all juveniles but failed to prevent the crossing of agile frogs and tree frogs (Figure 4). In case of the toads tested, larger individuals succeeded in crossing at a greater fence height (Figure 6). As expected, the addition of a 10 cm overhang (Crt+) increased the efficacy of the concrete fence, lowering effective fence height (H_{eff}) for the adults of all species where an overhang was tested, albeit, not for agile frogs (Figure 4). Hence, in case of most European amphibian species, road managers are left with the choice of a greater concrete fence height without overhang or a lower fence height with overhang. However, while an increase in fence height to, for example, 50 cm, may stop agile frogs, this will unlikely stop American tree frogs, unless an overhang is also installed. Accordingly, when more nimble

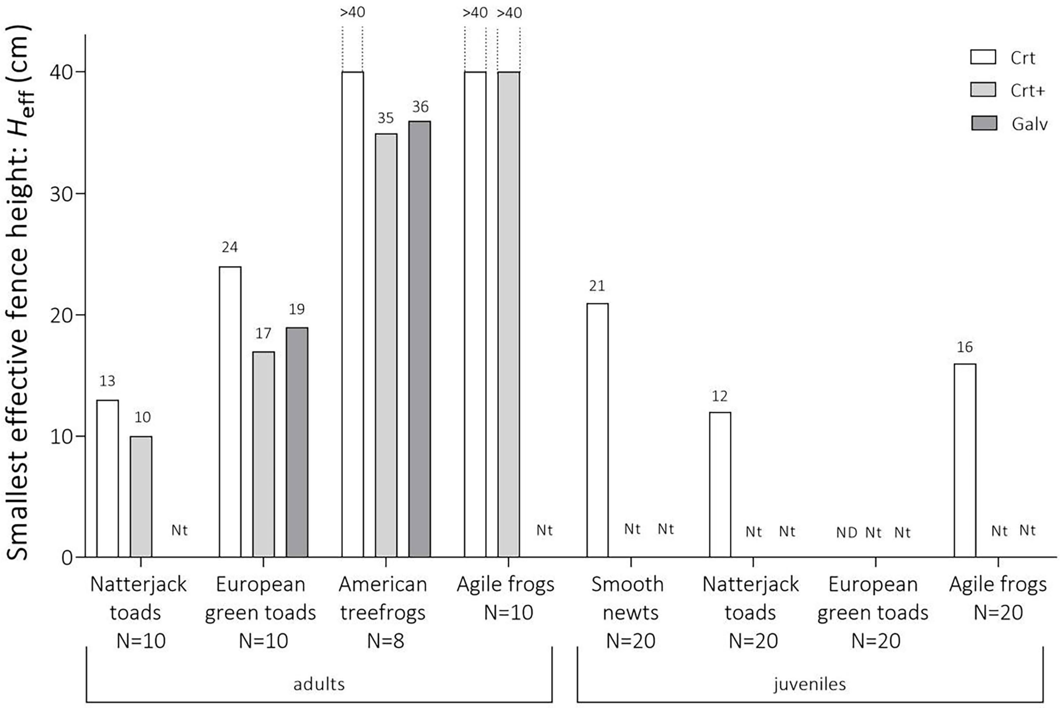

FIGURE 4
Smallest effective fence height (H_{eff}) for the amphibian species/developmental stages tested that prevented them to cross over various fence types, as indicated by different bar colors [white: simple concrete fence (Crt); light gray: concrete fence with a 10 cm horizontal overhang (Crt+); dark gray: galvanized fence (Galv)] with a bent overhang. Note that not all fence types could be tested with all species/developmental stages, as indicated by 'Nt' (not tested). Juvenile green toads never attempted to cross the fences tested, so that we could not determine H_{eff} in that case, as indicated by ND (not determined).

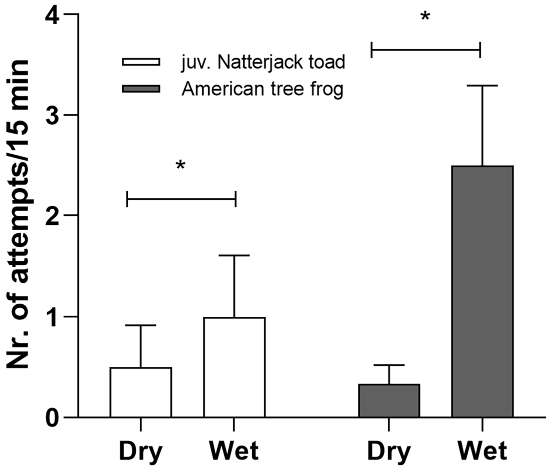

FIGURE 5
Number of crossing attempts per 15min periods for two climbing species: juvenile Natterjack toads (N=20) and adult American tree frogs (N=8). Both species preferentially used the wetted part of the fence (t=2,1, p<0.05).

species (agile frogs, tree frogs) are present, we strongly recommend the addition of an overhang to a fence. Such addition stopped the American tree frog, a species able to climb trees (Gourevitch and Roger Downie, 2018), at a fence height of 36 cm. Unfortunately, we were unable to test a fence height greater than 40 cm, so H_{eff} for agile frogs remains unknown, as they jumped across the greatest height tested, even in the presence of an overhang. This is likely of relevance for all proficient jumping amphibian species. Similar to previous recommendations (Morand and Carsignol, 2019), we therefore suggest a fence height of a least 50 cm with the addition of a horizontal overhang. The width of the overhang is crucial and anything less than 10 cm might not work well for frog species (Conan et al., 2022).

When the installation of a concrete fence is not possible (due to high costs and/or the destructive nature of its construction), road managers have the option to install other fence types. These might be less expensive to install but might also be less durable. As a first choice, road managers often consider the use of wire-netting fences to stop small animals. However, they are fragile and, most importantly, useless in preventing the crossing of green frogs, European green toad and many small mammalian species (Conan et al., 2022). A better option might be the use of galvanized metal fences of the type we tested (i.e., with a 5 cm horizontal overhang and a 2 cm downward component; Figure 3). Unfortunately, we were only able to test this fence type with green toads and tree frogs but H_{eff} was similar to that of the concrete fence with overhang (Figure 4). However, the overhang was problematic for the tree frogs, since they could attach themselves to the downward bent and then cross over the fence, reducing the efficacy of this fence type. Unfortunately, the 2 cm downward bent of the overhang was a structural requirement for the prototype we tested. Nevertheless, increasing the horizontal dimension of this overhang (e.g., from a width of 5 to 10 cm) might improve its efficacy and should be tested.

The 40 cm concrete fence stopped all juvenile amphibians, despite their similar locomotion mode to that of tree frogs (i.e., climbing),

TABLE 3 Morphological parameters determined and their relationship with H_{eff}.

Parameter	Species								Juveniles
	Green toad	r^2	p	Natterjack toad	r^2	p	Agile frog	American tree frog	
SVL (mm)	59.4 ± 2.2	0.24	0.18	53.7 ± 2.5	0.80	<0.001	52.5 ± 1.1	43.0 ± 0.8	~10
BM (g)	22.7 ± 2.2	0.3	0.13	17.5 ± 2.1	0.89	<0.001	21.3 ± 1.3	5.2 ± 0.1	NA
EL (mm)	153.6 ± 6.6	0.35	0.09	108.4 ± 6.9	0.79	<0.001	172.3 ± 3.3	128.1 ± 1.6	NA

Values are means ± SEM. Results of linear regression analysis (r2-and p-values) between a morphological parameter and effective fence height (H_{eff}) are included for species for which H_{eff} could be determined. Juveniles refers to all 4 species tested as juveniles; SVL = snout vent length, EL = elongation length, BM = body mass; approximate SVL is given for juveniles.

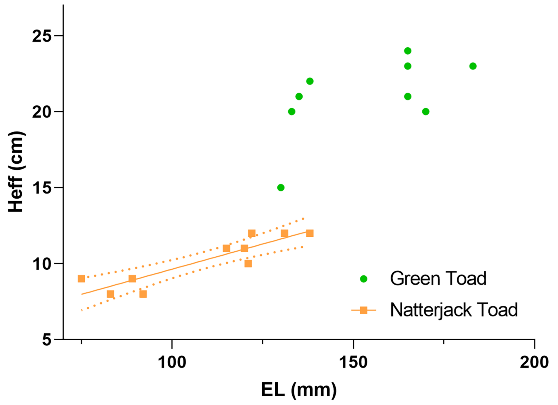

FIGURE 6
Relationship between elongation length (EL) and minimum effective fence height (H_{eff}) that prevented adult Natterjack and green toads from crossing the concrete fence without overhang (N=10 for both species). Significant relationships are indicted by the solid line, with dotted lines indicating the 95% confidence bands. There was a significant positive relationship between parameters for Natterjack toads (for EL: $p<0.001$, $r^2=0.80$), while this was not the case for green toads.

which were able to pass such a fence. Juveniles of all tested species were unable to reach a height greater than 10 to 20 times their body size (SVL ~1 cm). Unfortunately, we were unable to test the effect of an overhang with juveniles. Such structural addition will limit the presence of water along a fence during rainy weather (Schmidt et al., 2008), reducing fence wetting. The latter is of great importance, as fence wetting will likely ease fence crossing (Figure 5).

Our results are supported by previous studies indicating that durable and opaque structures are required to stop amphibians from crossing into roads and to guide them to the nearest wildlife passage (Brehme et al., 2021; Conan et al., 2022). A concrete fence equipped with a 10 cm overhang is an effective fence to stop the majority of amphibians present in Europe but only if it is well managed. In this context, we occasionally observed American tree frogs climbing the side walls of the arena, rather than the fence itself, from which they jumped over the fence, sideways. This is of great relevance for the situation on site. If the growth of sufficiently large/high vegetation near a fence is not prevented, tree frogs and similar climbing species will be able to overcome any type of fence. Hence, maintaining the vegetation near fences sufficiently small/low is essential to ensure the effectiveness of fences, especially in the case of climbing species (Speybroeck et al., 2018).

In our study we only addressed the situation of amphibian species. However, amphibians are not the only animals concerned by roadkill. Road mortality is also of concern for numerous other small animal species, such as small mammals, reptiles and also insects (Forman and Alexander, 1998; Dodd et al., 2004; Baxter-Gilbert et al., 2015). However, few studies have addressed the efficacy of fences for these groups (Dodd et al., 2004) but instead have focused on the efficacy of the ensuing tunnels to facilitate road crossing (Dodd et al., 2004; Glista et al., 2009). Furthermore, the lack of rigorous experimental testing of fences often leads to approximate measures by road managers which are based on practice rather than scientific evidence, and this includes the situation for amphibians (Morand and Carsignol, 2019).

In conclusion, a 40 cm concrete fence with a 10 cm overhang was effective to stop some of the amphibian species tested at their adult stage and all juveniles (Bufonidae, Hylidae). However, species with a great ability to jump were not stopped by such a barrier (e.g., *Rana dalmatina*). Accordingly, for the latter group of species we recommend a concrete fence height of at least 50 cm (see also Morand and Carsignol, 2019). If, for some reason, a full panel concrete fence cannot be used, we advise the use of a full panel galvanized metal fence with a minimal height of 50 cm and an overhang of at least 10 cm.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All procedures were carried out after obtaining the authorizations for capture and transport (2019-DREAL-EBP-0031) and a certificate for the detention of the various species in captivity (DDPP67-SPAE-FSC-2019-04). The experimental protocol was approved by the French Ethical Committee (CREMEAS) and the French Ministry of Environment: 'Ministère de la Transition Écologique' under agreement number (APAFIS #18546-2019011810282677.v7).

Author contributions

AC, JJ, and YH conceived the study and designed the methods. AC, MLB, ND, and LG collected the data. AC analyzed the data. AC drafted the manuscript with contributions from MLB, ND, LG, JF, ME, JJ, and YH. All authors approve this version of the manuscript for publication. They agree to be accountable for the aspects of the work that they conducted and will ensure that any questions related to the accuracy or integrity of any part of their work are appropriately investigated and resolved.

Funding

This study was conducted within the framework of the CERISE project and was funded by the French Minister of Ecology (DREAL Grand-Est), the Region Grand-Est, the European collectivity of Alsace (CeA), and the Eurometropole of Strasbourg (EMS). The funders had no role in any aspect of the study and agreed to its publication.

Acknowledgments

We thank all the students who participated in this study, Sylvia Pardonnet for help with video analysis, and Pierre Uhlrich for construction of the experimental arena. Two anonymous reviewers and the editor are thanked for their valuable comments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 07 March 2023
DOI 10.3389/fevo.2023.1123292

Spatially prioritizing mitigation for amphibian roadkills based on fatality estimation and landscape conversion

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SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 13 December 2022
ACCEPTED 14 February 2023
PUBLISHED 07 March 2023

CITATION
Gonçalves LO, Brack IV, Zank C, Beduschi J and Kindel A (2023) Spatially prioritizing mitigation for amphibian roadkills based on fatality estimation and landscape conversion. *Front. Ecol. Evol.* 11:1123292.
doi: 10.3389/fevo.2023.1123292

Roads cause biodiversity loss and the effects of wildlife-vehicle collisions may ripple from individuals and populations to ecosystem functioning. Amphibians are threatened worldwide and, despite being particularly prone to roadkill impacts, they are often neglected in assessments. Here, we develop a sampling and analytical framework for spatially prioritizing mitigation actions for anuran amphibian roadkills based on fatality estimation and landscape conversion. The framework is composed of the six following steps: (1) pre-selection of segments to survey using the wetland coverage in the surroundings and the presence of roadkills of aquatic reptiles as a proxy for wet areas; (2) spatiotemporally replicated counts with a dependent double-observer protocol, that is, each segment is sampled multiple times by two pairs of people on foot; (3) extraction of covariates hypothesized to affect spatial and temporal variation in roadkill rates and persistence; (4) hierarchical open-population N-mixture modelling to estimate population dynamics parameters, which accounts for imperfect detection and spatiotemporal heterogeneity in removal, detection, and roadkill rates, and explicitly estimates carcass entries per time interval. (5) Assessment of land cover transition to infer landscape stability; and (6) prioritization of segments based on higher fatality rates and lower landscape conversion rates. We estimated a mean of 136 (95%CrI=130–142) anurans roadkill per km per day in the 50 sample sites selected. The initial number of carcasses had a positive relationship with the percentage occupied by wetlands and a negative association with the percentage occupied by urban areas. The number of entrant carcass per interval was higher in the presence of rainfall and had a positive association with the wetlands cover. Carcass persistence probability was higher at night and lower in sites with high traffic volume. Ten segments (~1% of road extension) were prioritized using the median as threshold for fatality estimates and landscape conversion. It is urgent to appropriately evaluate the number of amphibians roadkilled aiming to plan and implement mitigation measures specifically designed for these small animals. Our approach accounts for feasibility (focused on sites with greater relevance), robustness (considering imperfect detection), and steadiness (less prone to loss of effectiveness due to landscape dynamics).

KEYWORDS

mitigation prioritization, anurans, imperfect detection, persistence probability, landscape transition, hierarchical models

Introduction

Road infrastructures are an important source of biodiversity loss and are spreading across the globe (Meijer et al., 2018; Wenz et al., 2020). Besides the increase in environmental degradation and the decrease in ecological connectivity, direct removal of individuals by roadkill can be a major cause of local population decline (Fahrig and Rytwinski, 2009).Road effects on wild populations can ripple to other levels of ecological organization, affecting ecosystem functioning (van der Ree et al., 2015). To mitigate efficiently the negative impacts of roads on wildlife, sound knowledge on where deaths are concentrated is fundamental (Gunson and Teixeira, 2015 but see Teixeira et al., 2017), as well as accounting for landscape stability to ensure that proposed measures are long-term lasting (Zeller et al., 2020). Generating such high-quality information can be challenging given the usually large extent of road networks, scarce time and financial resources available for field work.

It is possible to assess roadkill patterns based on road features, landscape characteristics, and species occurrence (Patrick et al., 2012; Girardet et al., 2015; Visintin et al., 2016). Nevertheless, probably the best information to assess where roadkills concentrate along a road is still by estimating fatalities based on observation/counts of carcasses. However, fatality estimations using raw counts can be biased, as observers might not detect all available carcasses. Moreover, removal of carcass from the road by scavengers or traffic may also be an important factor affecting estimates, especially when smaller animals are the study target (Santos et al., 2011; Villegas-Patraca et al., 2012; Barrientos et al., 2018; Schwartz et al., 2018). Not properly addressing these sources of error (detection and removals) in fatality assessments might produce biased roadkill estimations. As both errors may vary in space and time, their spatiotemporal heterogeneity should also be accounted for. If such aspects are not assessed, mitigation actions may be proposed at less effective sites.

Usual approaches to estimate wildlife fatalities at man-made infrastructures, when considering the sources of error, commonly use *ad-hoc* formulas based on detection rates and carcass removal trials from experiments or from comparisons with an assumed perfect-detection method (e.g., surveys on foot; Simonis et al., 2018; Teixeira et al., 2013). However, there have been claims for the application of process-based approaches while accounting for imperfect detection in carcass observations (e.g., wind farms, Péron et al., 2013; roads, Guinard et al., 2012). Such process-based approaches, typically based on open population capture-recapture models, tend to represent more accurately the dynamics of carcasses entering and leaving the sampled road segment (Guinard et al., 2015; Péron, 2018).

Even when roadkill hotspots are robustly estimated, mitigation measures can result in resource wasting if populations are locally affected due to other anthropic pressures. The effectiveness of an installed mitigation structure could rapidly decline in regions where anthropogenic landscape changes are more pronounced, causing shifts in the distribution and movement patterns of a species along a road. Hence, habitat stability is an important aspect to be included in the spatial prioritization of mitigation structures, especially when planning long-term measures (Clevenger and Ford, 2010; Zeller et al., 2020).

While often neglected in road fatality assessments, amphibians are one of the most affected taxa by roadkills, representing more than 90% of the fatalities in some cases (Fahrig et al., 1995; Glista et al., 2008; Coelho et al., 2012; Silva et al., 2021). Amphibians are the most threatened vertebrate group with 41% of the species at risk of extinction (IUCN, 2021). Their life cycle, with most species presenting an aquatic larval phase, imposes to adults and juveniles challenges in arriving and leaving water bodies every reproductive season. This can increase road encounter probability by amphibians and the potential negative population-level effects, especially for anurans species with lower reproductive rates, smaller body sizes, and younger ages at sexual maturity (Rytwinski and Fahrig, 2012). Moreover, their small size results in low detection and fast removal (Teixeira et al., 2013; Pereira et al., 2018) demanding sampling on foot and with short time intervals between occasions, which would represent a challenge for the survey of extensive road networks.

Here, we develop a framework for prioritizing road segments for amphibian roadkill mitigation based on fatality estimation and landscape transition (Figure 1). Our framework is composed of the six following steps:

1. Pre-selection of road segments with higher potential occurrence of amphibians;
2. Spatiotemporally replicated carcass count surveys by dependent double observers on foot;
3. Extraction of covariates that may influence the spatial and temporal variation in roadkill rates and persistence;
4. Fatality estimation with hierarchical modelling, taking into account imperfect detection and spatiotemporal heterogeneity in persistence and roadkill rates;
5. Landscape conversion using a transition rate from native to non-native land covers;
6. Road segment prioritization using higher roadkill rates and lower landscape transition rates as criteria.

We applied this framework to define priority segments for mitigation of amphibian fatalities on two roads surrounded by a mosaic of grasslands, pastures, wetlands, rice fields, and urban areas in southernmost Brazil. In the fatality estimation step, we used dynamic N-mixture models to evaluate the influence of land use and cover on the distribution of carcasses, the impact of raining on roadkill, traffic volume and day/night time on carcass persistence. We expected that wetlands and urban areas in the surroundings of a segment would have a positive and negative influence, respectively, on the spatial distribution of carcasses; that fatality rates would be higher in rainy occasions; that at segments with higher traffic volume, carcass would persist less; and that carcass persistence would be higher at night.

Materials and methods

Study area

Located in southernmost Brazil, the roads ERS-040 and ERS-784 have 84 and 15 km of length, respectively (Figure 2). The ERS-040 is surrounded by a heterogeneous landscape, with a mosaic of wetlands, urban areas, rice field and cattle ranching, while the ERS-784 is bordered by extensive exotic *Pinus* sp. plantations, scattered human occupation and wetlands. As the roads are designed to access the

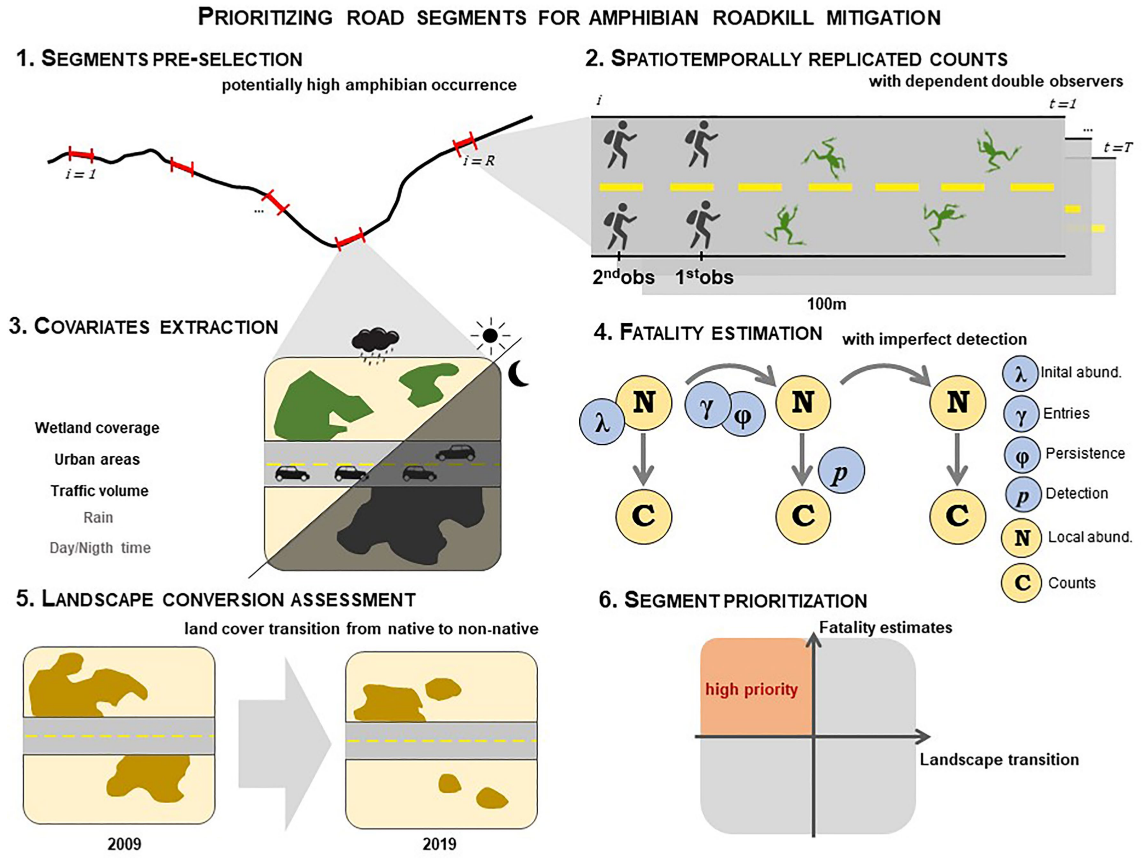

FIGURE 1
Framework to spatially prioritize road segments for amphibian roadkill mitigation based on fatality estimation and landscape stability. **(1)** R road sites of 100m length, where amphibian occurrence is expected to be higher, are pre-selected for sampling; **(2)** Each site i is surveyed T times searching for carcasses using a dependent double-observer protocol; **(3)** Covariates hypothesized to influence spatial and temporal variation in roadkill rates and persistence are compiled (black and gray letters indicate spatial and temporal covariates respectively); **(4)** Carcass counts C and covariates are fitted under a hierarchical open N-mixture model to explicitly estimate roadkill rates. Dynamics in the population of carcasses N is modelled as a result of two processes, entries and persistence. A roadkill rate is derived from the entry parameter γ; **(5)** Landscape stability is assessed by measuring the transition rates from native to non-native covers; and **(6)** Priority segments for mitigation are defined based on high fatality rates and low landscape transition rates.

coast, there is a strong increase in the daily traffic volume during the spring and more markedly in the summer months, that coincide with high amphibian activity.

Segments pre-selection for sampling

We identified road segments where we expected a higher concentration of amphibians (Figure 1.1). In the context of this study, the vast majority of amphibian species in the region are dependent on lentic environments (such as temporary and permanent pools, swamps, the edge of lagoons) and/or need these areas to complete their reproductive cycle. Hereafter, we will refer to these areas as wetlands. We selected 50 road segments with 100 m length using two sources of information: (i) percentage occupied by wetlands coverage in the surroundings obtained from remote sensing and field checking; and (ii) number of observed fatalities of aquatic reptiles (unpublished data obtained from a systematic survey of reptiles by car from the road concessionaire). Each segment was adopted as our sampling unit (site) and their extent was selected considering displacement capacities/willingness of amphibians along a fence (Duellman and Trueb, 1994; Brehme et al., 2021).

We calculated the percentage occupied by wetlands in a 1 km buffer centered on each segment, using two different classification sources followed by the inspection of high-resolution images using Google Earth Pro: the index of flooded surface in wetlands (Water In Wetlands-WIW- Lefebvre et al., 2019) and the Vegetation Coverage Map of Rio Grande do Sul - base year 2015, with 1:250,000 scale (Hoffmann et al., 2015). The WIW index was obtained through Google Earth Engine platform using Sentinel-2 satellite images available for spring and summer period (from September 2019 to January 2020), and the median of the pixel values between the dates obtained for the near-infrared (B8A) and short-wave infrared (B12) bands. To facilitate the identification of the wetlands, we modified the cut-off threshold of the B8A band to the value of 2000 nm and excluded the areas of rivers and deep lagoons. The WIW result is a raster in which each pixel with water on the surface received value 1 and the others value zero. We overlapped the WIW map with the classes water bodies, wetland, and wet grasslands from the Vegetation Coverage Map of Rio Grande do Sul. With this final map, we selected

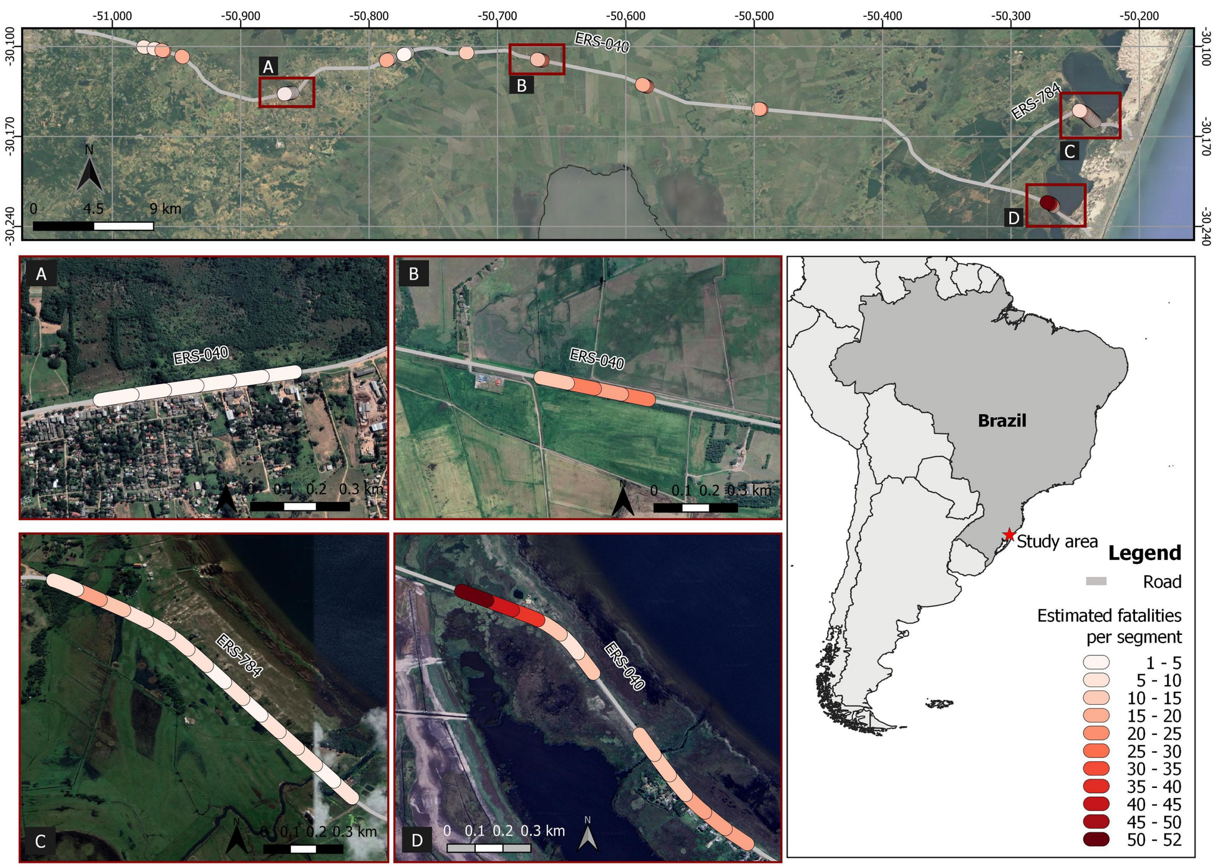

FIGURE 2
Road segments (100m length) sampled for amphibian fatality estimation in southernmost Brazil (ERS-040 and ERS-784 roads) aiming at identifying priority locals for mitigation. In the upper image, colored circles represent estimated fatalities for the sampled segments, from which some of them (framed by squares) are shown in detail from **(A–D)**. Map data © OpenStreetMap contributors, 2022.

road segments with more than 30% of wetland within the 1 km buffer centered on each segment. We also selected segments based on the presence of at least two fatalities of aquatic reptiles (EGR, 2020). We use the occurrence of aquatic reptiles as an indicator for wet areas that could not be detected by remote sensing. We specifically considered aquatic snakes that feed on amphibians (water snake, *Helicops infrataeniatus*; and green snake, *Erythrolamprus poecilogyrus*) and turtles.

Carcass surveys

We conducted spatiotemporally replicated counts with a dependent double-observer protocol, that is, each of the 50 selected 100 m segments was sampled multiple times by two pairs of people on foot (i.e., front-pair is the first observer and the back-pair is the second; Figure 1.2). Each member of the front pair and back pair sampled one road lane and its respective shoulder. Surveys occurred twice a day – one survey at dawn and one at dusk – during three consecutive days in January of 2021, resulting in six sampling occasions (i.e., visits) per site. The first observer of each lane walked ahead and recorded all possible carcasses and the second walked behind and only recorded the carcasses not detected by the first (i.e., dependent double observers; Figure 1.2). Detected carcasses were not removed until the last sampling occasion was finished. For each detected carcass, we took a picture, recorded date, occasion, observer (1st or 2nd) and segment ID. Each anuran carcass record was identified, whenever possible, on the field, or based on pictures or carcasses collected after the last sampling occasion. We did not estimate fatality numbers per species since our aim was to exemplify a general application of the framework for amphibian roadkill estimation.

Covariates extraction

We included five covariates to estimate fatalities accounting for imperfection detection and its spatiotemporal heterogeneity (Figure 1.3): (i) wetland coverage, (ii) urban areas coverage, (iii) traffic volume, (iv) the presence/absence of rainfall, and (v) day/night time. We obtained the percentage occupied by wetland coverage and urban areas in 200 m buffers centered on each road segment. Wetland coverage was obtained from the same classification used for the site pre-selection. Urban areas were manually classified based on a 2019 high resolution image using Google Earth Pro, considering polygons encompassing each edification and human settlement within the 200 m buffer. We categorized the traffic volume into three levels (low, medium, and high) based on the proximity to the populous human settlements and the traffic distribution along a road corridor from populous cities to the coast. Higher traffic was considered for all segments located in the most populous city (Viamão) which is the connection to the main city in the region (Porto Alegre); medium traffic segments were in an agricultural area that connects the studied road to a federal road; and lower traffic segments were in the end of the road that have access to two coastal cities (Cidreira and Balneário Pinhal). The presence/absence of rain in the previous interval for each occasion was based on recordings of rainfall between 6 am and 5 pm for dusk sampling occasions and between 6 pm and 5 am for dawn occasions. Rainfall data were obtained from the closest weather station for each site: Tramandaí (code A834 from the Brazilian National Institute of Meteorology) and Viamão (code 432300202A from the National Center for Monitoring and Natural Disaster Alerts; CEMADEN, 2022; INMET, 2022). Each interval between occasions was defined as nighttime if sampling visit occurred during dawn and as daytime if occurred at dusk.

Dynamic N-mixture model for fatality estimation

We applied a dynamic N-mixture model to the double-observer carcass counts in each visit $t \in \{1,\ldots,T\}$ for each site $i \in \{1,\ldots,R\}$ (Figure 1.4), derived from the Dail-Madsen formulation with a robust design used for living populations (Dail and Madsen, 2011; Zhao and Royle, 2019). This model assumes that the local carcass population size varies throughout the visits as a result of two dynamic parameters: (i) carcass entries per interval; and (ii) carcass persistence probability between two visits so that $N_{i,t+1} = S_{i,t} + E_{i,t}$, in which $S_{i,t}$ is the number of remaining carcasses from the previous visit and $E_{i,t}$ is the number of entering carcasses in the previous interval. The population size of the first visit $N_{i,1}$ is estimated using a Poisson distribution with mean (and variance) λ. The number of remaining carcasses $S_{i,t}$ is assumed to be a result of a binomial distribution in which each carcass from the population $N_{i,t}$ has a probability φ to persist until the next visit. The number of entering carcasses $E_{i,t}$ is assumed to follow a Poisson distribution with mean and variance γ. In the observation process, as surveys were conducted with a dependent double-observer protocol, the counts $C_{i,j,t}$ of each pair of observers $j \in \{1,2\}$ are assumed to follow a multinomial distribution. Then, each carcass available on the population $N_{i,t}$ has a probability *p* of being detected by the first pair of observers and a probability $(1-p)p$ of being detected by the second pair. Spatial and temporal variation (i.e., heterogeneity) in the four basic parameters (λ, φ, γ, and *p*) can be modelled as linear functions of covariates using the corresponding link functions (logit for probabilities and log for Poisson).

This approach permits to explicitly derive roadkill rate estimates (i.e., number of entering carcasses per interval), while taking into account imperfect detection and spatiotemporal heterogeneity in all parameters. Furthermore, it has the advantage of not requiring marking individual carcasses, neither trial experiments to separately estimate persistence and detection.

We considered effects of wetland coverage and urban areas on the initial carcass abundance (number of carcasses in the first occasion); wetland coverage and rain on entrant carcasses; and traffic volume and day/night time on carcass persistence. Carcass detection probability by each pair of observers was considered as constant. We estimated, as a derived parameter, an average roadkill rate per segment ($Fatalities / day$) by calculating the mean number of entrant carcasses between the first and the last occasion and an overall roadkill rate for all segments $\left(Fatalities / (km.day)\right)$. We fitted the carcass count data to the dynamic N-mixture model under a Bayesian approach using software JAGS (Plummer, 2003) accessed from the package *jagsUI* (Kellner, 2015) in R (R Core Team, 2022). We ran three parallel Monte Carlo Markov Chains with 10,000 steps in the adaptive phase, followed by 100,000 steps from which the first 20,000 were discarded. This resulted in 240,000 samples of the posterior distribution from which we calculated the mean and 95% credible intervals for each parameter. We assigned vague prior distributions for all estimated parameters. Model convergence was assessed by visually inspecting the chains' traceplots and using the R-hat statistics (R-hat ≤ 1.1). R and JAGS code are provided in Supplementary material 1.

Landscape conversion

To determine landscape stability for each segment, we used a landscape transition metric based on land cover transitions from native to non-native (Figure 1.5). The segments with lower transition rates represent sites that had a lower conversion of their surrounding landscape, and we assumed they are more prone to be stable over the long term, and thus are more suitable to receive mitigation actions that are fixed in space, such as fences associated to underpasses. We defined a 200 m buffer centered on each segment to extract the land-cover map to calculate the landscape transition rate. We extracted the maps for the years of 2009 and 2019 from the Mapbiomas V5.0 (Souza et al., 2020) and reclassified, grouping them into two classes: native and non-native land covers, using the software QGIS V3.12 (QGIS.org, 2022). With the *Dinamica EGO* software (Soares-Filho et al., 2009), we obtained the transition rate for each segment buffer by calculating the proportion of native land cover in 2009 that became non-native in 2019. We have considered here that this stability in landscape conversion would indicate areas in which mitigation measures would last longer because the region where the roads are embedded has a consolidated historical land use. However, we note that in different contexts, other criteria may be used.

Segment prioritization

We used a four-quadrant prioritization matrix to select segments for mitigation, considering: (i) the highest estimated fatality rates and (ii) the lowest transition rates from native to non-native land cover on the surrounding landscape (Figure 1.6). Quadrants were delimited by the median of the estimated fatality rates and the land-cover transition rate. Hence, road segments located in the quadrant formed by values above the median of estimated fatality rates and below the median of land-cover transition rate are the high-priority segments for mitigation.

Results

We found amphibian carcasses in 49 of the 50 sampled segments in at least one of the six occasions. Maximum count per visit in these

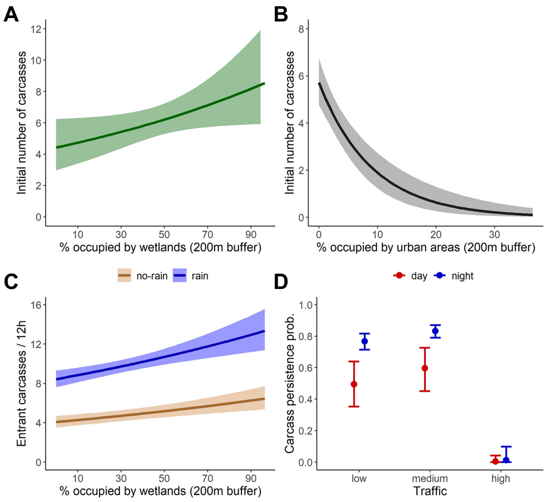

FIGURE 3
Predicted relations from the dynamic N-mixture model applied for dependent double-observer counts of amphibian carcasses. Relationship of the initial number of carcasses with **(A)** wetlands coverage, and **(B)** coverage of urban areas; **(C)** the number of entrant carcasses with wetlands coverage for rainy and not-rainy intervals; and **(D)** the carcass persistence probability with traffic volume and time of the day. Shaded areas and error bars represent the 95% credibility intervals.

TABLE 1 Coefficient estimates obtained from the dynamic N-mixture model applied for dependent double-observer counts of amphibian carcasses.

	Estimate	Std. error	−95%CL	+95%CL
Initial abundance (λ)				
λ (Intercept)	1.48	0.19	1.09	1.83
Wetland	0.69	0.35	0.02	1.40
Urban	−11.11	2.12	−15.51	−7.24
Carcass entries (γ)				
γ (Intercept)	1.41	0.07	1.26	1.55
Wetland	0.48	0.11	0.25	0.70
Rain	0.73	0.06	0.60	0.86
Persistence (φ)				
Traffic low (Int)	1.19	0.15	0.92	1.50
Traffic medium (Int)	1.61	0.15	1.33	1.91
Traffic high (Int)	−4.33	1.37	−7.66	−2.21
Day	−1.22	0.15	−1.52	−0.93
Detection (p)				
p (Constant)	0.69	0.1	0.66	0.72

Wetland = proportion of wetlands (200 m buffer around segment); urban = proportion of urban areas (200 m buffer around segment); rain = presence of rainfall in the interval; traffic = classification of traffic category for segments; day = daytime, nighttime is fixed at the intercept. The detection parameter p is in the probability scale.

segments varied from one to 127 carcasses detected by the two pairs of observers, while the mean count for all segments was 12 carcasses. Hylidae and Leptodactylidae families represented 75% of carcasses identified, whereas *Dendropsophus* spp. and *Leptodactylus luctator*, *Leptodactylus gracilis*, and *Pseudis minuta* were the most recorded species (Supplementary material 2).

The estimated average roadkill rate at the 50 segments during the 3 days was 136 (95%CrI = 130–142) amphibian fatalities per km per day. Mean roadkill rate for the 100 m segments ranged from 1.3 (95%CrI = 0.4–3.2) to 52.7 (95%CrI = 48.4–57.6) fatalities/day (Figure 2). We found a positive relationship of the initial carcass abundance with wetlands coverage (Figure 3A) and a negative relationship with urban areas coverage (Figure 3B). The number of entrant carcasses per interval was positively influenced by the wetland coverage and was twice higher when rain occurred (Figure 3C). Carcass persistence was higher during the night and very low for segments with high traffic volume (Figure 3D). The probability of each pair of observers to detect an amphibian carcass was 0.69 (95%CrI = 0.66–0.72; Table 1).

Landscape transition rate varied from 0 to 98% of conversion. The median of the estimated fatality rate was 9.99 individuals per day and the median of the landscape transition rate was 5% in a 10-year interval. Ten segments were prioritized to receive the mitigation actions with these thresholds for fatality estimates and landscape stability, meaning a 5-fold reduction of segment numbers (Figure 4).

Discussion

Although amphibians are often a major group affected by roadkill (Fahrig et al., 1995; Glista et al., 2008; Coelho et al., 2012), planning efficient mitigation actions for this group imposes challenges given the usual difficulty to survey their carcasses. In order to obtain reliable estimates of amphibian roadkill rates and propose enduring mitigation measures, we present a prioritization framework based on a pre-selection of segments to be surveyed, an explicit modeling of fatalities with imperfect detection, and an evaluation of landscape stability. With this approach, we were able to identify 10 high-priority 100 m-segments to receive mitigation measures, i.e., 1 km in a context of about 100 km of road.

By choosing segments more likely to concentrate amphibian roadkills, we have reduced to 5% the length of road to be sampled. The usual small size of amphibians makes them hard to detect from traditional carcass survey methods (i.e., by car). By searching amphibian carcasses on foot, we obtained a carcass detection probability of ~70% by each pair of observers, which we considered quite good for this group. Moreover, the spatiotemporally replicated design proposed here requires carrying out counts in multiple visits at the segments, making more difficult to cover a large road extent. Therefore, given the logistic constraints to survey amphibian carcasses on foot at extensive roads, pre-selecting segments based on habitat features is a way to make sampling more feasible. Importantly, the pre-selection criteria must be chosen according to the habitat associations of each target group.

Spatiotemporally replicated counts of carcasses can be a cost-effective method to robustly estimate roadkill patterns. Fitting these counts with dynamic N-mixture models enables to explicitly derive

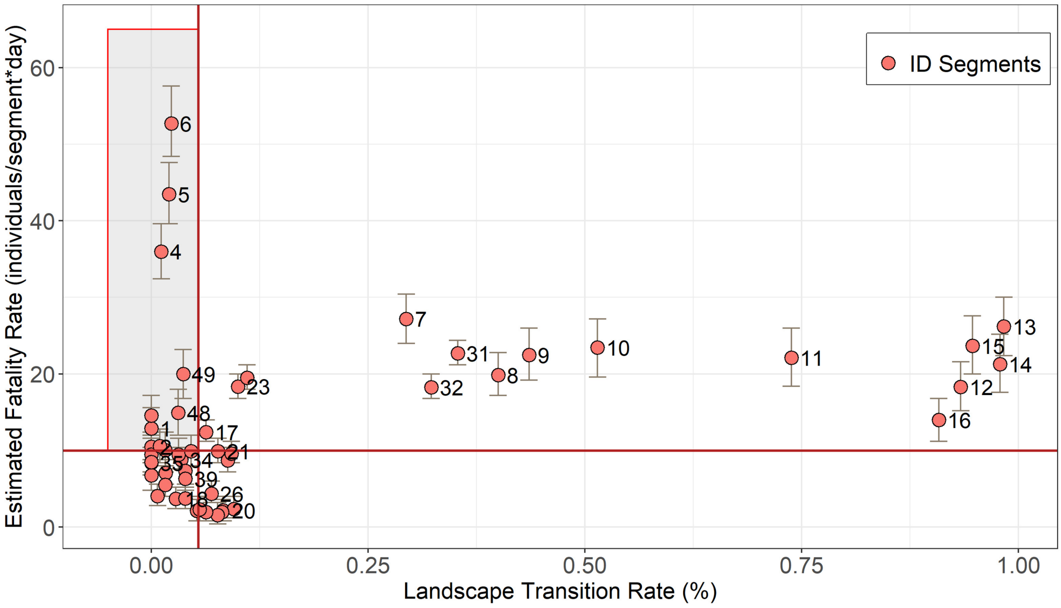

FIGURE 4
Segment prioritization to receive mitigation actions for amphibian roadkills, based on fatality estimation and landscape stability. The red lines are the threshold defined by the median values of the land-cover transition rate (5% of landscape transition) and the fatality estimate (9.99 individuals per day). Priority segments are within the shaded box. Numbers correspond to segment IDs and bars to the credibility intervals of fatality estimates (Supplementary material 3).

roadkill rates for known time intervals (time between visits), while formally accommodating the potential sources of error (and their heterogeneity) in the same modelling structure. Previous studies have also used carcass counts or occurrences split into segments to model spatial variation on roadkills and identify hotspots based on a Poisson distribution (e.g., Santos et al., 2017; Lin et al., 2019). However, such approaches fail in accounting for imperfect detection (and even less the possibility of modeling the heterogeneity in persistence and detection) and do not provide reliable explicit rates of roadkills. Our directly derived roadkill rates also have the advantage of being comparable among studies, species or regions. Sources of errors (persistence and detection) in roadkill assessments are commonly addressed using trial experiments in which a known number of carcasses is disposed on the road (Barrientos et al., 2018; Gonçalves et al., 2018). However, there might be spatial and temporal variations in persistence and detection that are unfeasible to capture and represent with experiments. For example, as we found here, carcass persistence presented considerable variations according to the traffic volume at the segment and the period of the day. Not accounting in trial experiments for such heterogeneities may produce biased estimation of roadkill patterns. Some studies have made attempts to use hierarchical models in the context of roadkill data to identify priority segments while accounting for imperfect detection (e.g., Santos et al., 2018; Hallisey et al., 2022). Nevertheless, the approach adopted in these studies uses detection/non-detection data and makes inferences on "carcass occupancy" for a wide-time window, and not roadkill numbers.

Revealing effects of spatial and/or temporal covariates in the roadkill rates can be useful, for example, to predict fatalities hotspots in roads planned to be constructed or to plan carcass surveys in moments that patterns would be more highlighted in data. As expected, we found here that wetland coverage (high-quality habitat for most amphibian species in the studied region) in the road surroundings influenced the mean number of amphibians roadkilled. Segments with 90% of wetland coverage can present on average 47% more fatalities than segments with 10%. Such identified relationships could be applied to predict segments with potential higher roadkill rates in other roads with similar landscape characteristics. Moreover, the presence of rain during the interval between sampling visits resulted in about twice higher amphibian fatalities than intervals without rain. When planning carcass surveys, this kind of temporal variation should be taken into account to prioritize periods that might maximize the detection of spatial patterns in roadkill rates.

One advantage of the modeling approach we used is that it allows ecologists to estimate the dynamic parameters without marking individuals (Dail and Madsen, 2011; Dénes et al., 2015), as it was proposed by Péron et al. (2013). An important assumption of capture-recapture models is that marked and unmarked individuals have the same persistence and detection probabilities. By fitting carcass count data with N-mixture models, we avoided the need of marking the small amphibian carcasses, procedure that is logistically difficult and could, for example, influence later detections or affect the persistence of carcasses that were adhered to the substrate. However, because of the lack of information on individual capture histories, dynamic N-mixture models could sometimes present problems in parsing out entry and persistence processes (Kéry and Royle, 2021). Despite this issue may result in biased absolute roadkill estimation, this approach is still useful in relative terms (i.e., which sites present higher roadkill rates) to identify priority sites for mitigation. One alternative to ensure unbiased absolute roadkill estimations is to mark just a few individuals to directly inform entry and persistence processes.

Our framework recognized the priority road segments for amphibian mitigation not only identifying at which places animals tend to die more, but also including landscape conversion as a long-lasting criterion. We highlight the importance of considering the endurance of mitigation actions jointly with higher roadkill rates to ensure effective measures in long-term. Other criteria than lower landscape conversion rates could be used depending on the context of the surrounding landscape, such as where roads which are built in pristine areas (e.g., Amazonian Arc of Deforestation). Moreover, depending on the mitigation objectives, prioritization could consider other criteria, such as the number of threatened species per site, or *per capita* mortality, if cascading population effects are the main concern (Teixeira et al., 2017). When data on the population in the surroundings are available and when the maintenance of a population is a conservation target, this kind of information can provide important assets to use in the prioritization step. There have been cost-effective manners of obtaining population data for one or more amphibian species in the surrounding of roads, in order to integrate information in mitigation planning, such as automatic acoustic recordings (Marques et al., 2013) or citizen science programs that monitor amphibian migrations [e.g., Toads on Roads (Petrovan et al., 2020); Big Night programs (Sterrett et al., 2019)].

We proposed our sampling and analytical framework to estimate amphibian roadkill rates accounting for imperfect detection and its heterogeneity. The modeling approach can be expanded in order to accommodate other sources of variation, for example, differentiating species and including them as random effects in multi-species models (Yamaura et al., 2012; Dorazio et al., 2015). This might be particularly important when there is interest in threatened species. Also, although we have focused on amphibians, this framework could be useful to identify locals for mitigation measures for any roadkill target, especially when marking individuals is a difficult task.

Our study set out to better support mitigation prioritization decisions and such information can be used to inform where to implement road management actions. However, in terms of conservation, an imperative further step is to indicate and implement appropriate mitigation measures for road management to reduce roadkill of the target group studied here. Fencing is the most suitable spatial mitigation structure to maintain amphibians off the road and mitigate roadkills (Cunnington et al., 2014). Also, it is essential to promote safe crossings for amphibians daily movements with the implementation of wildlife passages (Woltz et al., 2008; Beebee, 2013; Jarvis et al., 2019). We highlight that the implementation of those mitigation measures is not the final step either since their effectiveness should be evaluated with robust designs aiming to collect the relevant evidence (Helldin and Petrovan, 2019; Ottburg and van der Grift Edgar, 2019; Schmidt et al., 2020).

In conclusion, our study developed a sampling and analytical framework to improve road management toward spatial prioritization of mitigation measures for amphibian roadkill. We proposed six steps to better support amphibian conservation decision making and such information can be used to inform where to implement road management actions. Our findings are also useful to plan future carcass surveys in locations and moments that patterns would be more suitable.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://figshare.com/s/0cebdfcedb591dea6fed.

Author contributions

All authors conceived the idea of the manuscript, designed the survey, interpreted the results, wrote sections in all drafts, contributed critically to the writing, and gave final approval for publication. IB developed the analytical approach. JB and CZ collected roadkill data. JB, CZ, and LG compiled and synthesized data. JB and IB performed data analyses. JB, CZ, and IB made figures and tables. LG and IB led the writing process, working on the final version of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

Financial support was provided by Fundação Empresa Escola de Engenharia da Universidade Federal do Rio Grande do Sul for LG, IB, CZ, JB, and AK (Projects FEENG/307 and FEENG/437). LG is funded by Programa USP Sustentabilidade da Superintendência de Gestão Ambiental da Universidade de São Paulo.

Acknowledgments

We thank STE – Serviços Técnicos de Engenharia S.A., EGR – Empresa Gaúcha de Rodovias, and Fepam – Fundação Estadual de Proteção Ambiental Henrique Luis Roessler for the opportunity to apply our framework in the mitigation planning of roads in the renewing process of the operating environmental license. We thank Guilherme Iablonoviski and Igor Pfeifer Coelho for the support in the land cover maps; Dener Heiermann and Maria Eduarda Bernardino Cunha for amphibian identification; and all survey teams for their effort: Ingridi Camboim Franceschi, Samuel Ferreira Gohlke, Priscila Cortêz Barth, Maria Eduarda Bernardino Cunha, Nikolas Raphael Oliveira Giannakos, Giulia Dorneles Barbieri de Campos, Isabel Salgueiro Lermen, Igor Oliveira Gonçalves, Maitê Zanella Busanello, Dener Heiermann, Érico Moreira de Miranda, and Deivid Pereira.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1123292/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 17 March 2023
DOI 10.3389/fevo.2023.1133253

Pollutant accumulation in road mitigation tunnels for amphibians: A multisite comparison on an ignored but important issue

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Underpasses or road tunnels are increasingly installed to reconnect habitats and ensure safe wildlife passage, thus preventing habitat fragmentation caused by roads and mortality from collisions with vehicles. In the UK, such underpasses are regularly implemented for amphibians and especially the protected great crested newt, *Triturus cristatus*. However, roads are also a key source of environmental pollutants including trace metals, road salt, petroleum and diesel hydrocarbons and these might impact road mitigation structures where amphibians are funnelled to, yet the extent and implications of such pollutants are almost never quantified in relation to this aspect, despite the recognised sensitivity of amphibians to chemical pollutants. Sediments were analysed from four amphibian road mitigation tunnel sites across the UK and compared to natural soil formations at local reference sites to determine whether contaminants were indeed accumulating within the tunnels. Three potential contaminants (copper, lead, and total petroleum hydrocarbons) were found in greater concentrations in the underpass sediments than respective reference sites at three of four locations, while one (zinc) was found in greater concentrations at all four studied underpasses compared to reference sites. Aggregated sediment pH value was significantly greater in the underpass sediment than the respective reference sites at all four study sites and in several instances the contaminants reached values that exceeded the thresholds of environmental concern. Despite the large geographic area covered and the significant site differences the absolute values of potential pollutants in tunnels were similar across sites, thus suggesting similar pollution sources and pathways. These results suggest road tunnels installed for ecological mitigation could be a significant pathway for pollutants from road surfaces to amphibians and it is recommended that focussed monitoring and maintenance of the underpasses is enacted given that their short or long-term impacts on amphibians are currently unknown. Potential management options could include regular jet-washing of the underpasses, or alternatively, pre- or post-implementation modifications of mitigation designs should aim to minimise the pollution pathway from road surfaces.

KEYWORDS

road mitigation, urban pollutants, road runoff, trace metals, de-icing salts, *Triturus cristatus*

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SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 28 December 2022
ACCEPTED 27 February 2023
PUBLISHED 17 March 2023

CITATION
White KJ, Petrovan SO and Mayes WM (2023)
Pollutant accumulation in road mitigation
tunnels for amphibians: A multisite comparison
on an ignored but important issue.
Front. Ecol. Evol. 11:1133253.
doi: 10.3389/fevo.2023.1133253

1. Introduction

Roads are well understood to cause complex and substantial negative impacts on global biodiversity and the research related to these impacts, often termed "road ecology," has developed significantly in the past decades in terms of documenting and understanding the consequences of wildlife traffic mortality and fragmentation of habitats, but also indirect impacts linked to noise, artificial light, and chemical pollution for a variety of taxa (Forman et al., 2003; Van Der Ree et al., 2015). Amphibians are often the focus of such studies as they can be relatively easy to study compared to other taxa but are also considered to be exceptionally at risk from road impacts including from direct road mortality due to their need to travel between terrestrial and aquatic habitats but also their vulnerability to pollution due to their more permeable skin (Beebee, 2013). Roads can create "road-effect zones," where their impact extends as much as 1 km beyond their physical boundaries and influences the abundance and distribution of amphibian taxa (Eigenbrod et al., 2009) due to a combination of mortality, fragmentation and indirect impacts of disturbance. Chemical pollution from road surfaces for instance impacts amphibians in multiple ways and creates substantial road zones in some areas due to road salt and can create behavioural, physiological and demographic consequences for amphibians through wetland salinisation affecting different life stages (Sanzo and Hecnar, 2006; Karraker et al., 2008; Hintz and Relyea, 2019). However, little comparative research focus has been directed towards understanding the impacts of other road pollutants in relation to amphibians, such as specific trace metals, probably due to the multifactorial nature of these pollutants (e.g., acting in combinations of substances, pH levels, terrestrial or aquatic, etc.) as well as the logistical complexity of many of the required analyses. Given the continuous global expansion of road networks and traffic and the increasing available volume of research and evidence from practical mitigation implementation, it is important to identify outstanding knowledge gaps that might hamper the overall aim of reducing the negative impacts of transport infrastructure on amphibians, including for road pollutant substances for which, in some cases, even basic availability data remains scarce.

In the UK, the great crested newt (*Triturus cristatus*) is the largest species of newt and a strictly protected amphibian (Langton et al., 2001) yet, despite undergoing substantial declines in the latter half of the 20th century due to habitat loss (especially ponds) and fragmentation from urbanisation and agricultural intensification, the species remains relatively widespread and can be locally abundant (Langton et al., 2001). The species was listed on Annex IV of the EC Habitats and Species Directive (English Nature, 2001; Griffiths et al., 2010) and legislation protects the species at all life stages, and their habitats, from reckless and intentional damage and destruction (English Nature, 2001; Langton et al., 2001; Natural England, 2015). In areas considered potentially suitable, it is therefore a legal requirement to check for *T. cristatus* populations before construction of any new developments to prevent adverse impacts on the species that might be sublethal or lethal and, if a population is discovered, then mitigate the anticipated adverse impacts. The level of mitigation required depends on the size and type of anticipated impact, and the size and importance of the population affected but may include changes in timings of construction works, translocating the population to an area of newly created habitat, or exclusion from the area of works (English Nature, 2001). Given that development impacts often mean newt habitat becomes fragmented by a road, one specific mitigation option is the installation of tunnels beneath roads, which enable the species to migrate safely from one side of a road to another, thereby preventing habitat and metapopulation fragmentation and direct road mortality caused by crossing roads (English Nature, 2001; Schmidt and Zumbach, 2008; Lesbarrères and Fahrig, 2012; Matos et al., 2017). Such tunnels or underpasses have been shown to be effective solutions in some contexts (Lesbarrères et al., 2004; Jarvis et al., 2019) and come in a variety of technical specifications, from simple concrete tubes and various dimensions of concrete box culverts, to polymer concrete surface tunnels (ACO-type), where the top part is embedded in the road surface and provisioned with small grid openings that allow temperature and humidity to balance between the outside environment and the inner part of the tunnel, something directly relevant as temperate amphibians are reluctant to use cold, dry and entirely dark tunnels, especially over distances exceeding 15–20 m in length (Schmidt and Zumbach, 2008; Matos et al., 2019). However, White et al. (2017) highlighted concern that road pollutants may be entering and accumulating in sediments inside these tunnel structures, potentially exposing migrating newts or other amphibians to the plethora of pollutants associated with roads and traffic. Broadly, these typically consist of total petroleum and diesel hydrocarbons, trace metals, de-icing salts, and plastic/rubber particulates from vehicle wear and tear (Ward, 1990; Bäckström et al., 2004; Ziajahromi et al., 2020). There are 11 elements that are strongly correlated with roads which show an increase in concentration with mean traffic density (Ward, 1990). Several of these elements are sourced from general vehicle wear; copper (Cu), cadmium (Cd), and zinc (Zn) are known to disperse from brake linings, brake pads and tyre wear, and Zn may additionally be sourced from the corrosion of galvanised safety barriers and similar structures (Ward, 1990; Legret and Pagotto, 1999). Some multielement contamination may also stem from fuel drips and spillages; as well as typical hydrocarbons, petroleum and diesel can also contain a range of different trace metals including Cu, Pb, vanadium (V), and Zn (Chu-Van et al., 2020). Additional diffuse sources of some elements include vehicle emissions, drips of crankcase oil, and wear of asphalt road surfaces (Brown and Peake, 2006). Furthermore, White et al. (2017) found evidence of pollution in mitigation tunnels associated with the tunnel structures themselves, with highly alkaline waters (pH >10) leaching from the cement used as mortar to link together the polymer concrete sections of the tunnels. At such high pH, mobility of oxyanionic elements such as chromium (Cr), can also pose environmental risks alongside the extreme pH itself (e.g., Gomes et al., 2016). However, this was based on survey data from multiple tunnels but within a single mitigation site in Northern England and additional data is required to understand the broader relevance and implications of these initial findings.

Due to their permeable epidermis and typically biphasic aquatic and terrestrial life stages, many amphibians are highly sensitive to environmental changes, and environmental pollution is a regularly documented cause of global amphibian declines (Hayes et al., 2010). If road pollutants are accumulating in the tunnel sediment which migrating newts are frequently in close contact with, it is possible that they could be exposed to sublethal or even lethal effects in an environment which was placed to protect them from harm (White et al., 2017). Exposure of amphibians to road pollutants *via* mitigation is important to verify given that amphibians are funnelled by people towards and into such structures using barrier fences, therefore concentrating numerous or most individuals from an area into narrow engineering structures. Ensuring such structures are safe to use by amphibians, both in terms of lethal and non-lethal impacts, should be an important element of road ecology and ecological mitigation yet, in practice such studies are virtually non-existent to our knowledge in relation to road pollutants.

This study undertakes a multi-site assessment to investigate whether mitigation underpasses are preferential sinks for road-based pollutants compared to nearby reference sites in the UK and to create a first stage assessment of the potential problems and impacts as well as opportunities for solutions and future prioritised research.

2. Methods

2.1. Study sites

Road mitigation tunnels at four study sites across the UK were chosen for sediment analysis. Three of the four sites (Site 1, Site 2, and Site 3: Figure 1) host known *T. cristatus* populations of medium – large size, based on the Great Crested Newt Mitigation Guidelines (English Nature, 2001) as well as other amphibians and other threatened vertebrates. Site 4 has several substantial amphibian populations including common frog, *Rana temporaria*, common toad, *Bufo bufo* and palmate newt *Lissotriton helveticus*, though no *T. cristatus* populations, and all of which are known to use the road mitigation tunnels (Hill et al., 2019).

Widely used polymer-concrete amphibian climate tunnels (also called surface tunnels or ACO-type tunnels) were installed at all four sites to enable individuals to safely navigate between ponds and terrestrial habitat to forage, breed and overwinter. The tunnels are approx. 0.5 m × 0.5 m and have open 6 × 3 cm slots along the road surface which lead down into the tunnels to enable light penetration during the day and humidity circulation inside the structures, to make the tunnels more attractive for individuals to use (Figure 1).

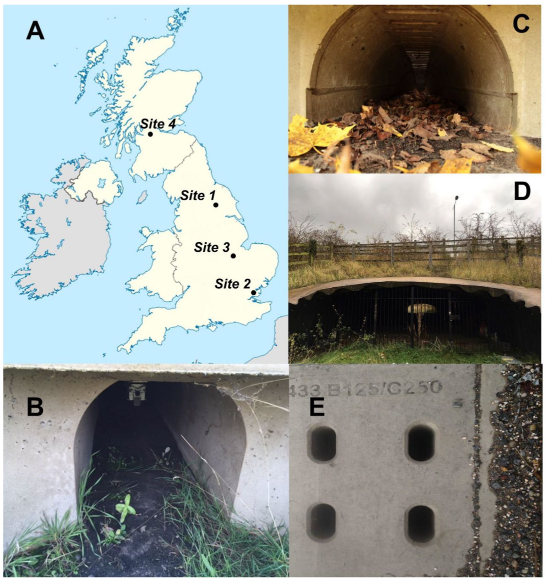

FIGURE 1
Site location **(A)** and example images of sample locations. **(B)** sediment build up in ACO tunnel entrance at Site 1; **(C)** ACO tunnel entrance showing grid at the top providing opening to the road surface above (Site 1); **(D)** larger concrete culverts – one of the two types of underpass installed at Site 3; **(E)** Road surface at Site 1 showing dust build up (right of image) and apertures in tunnel to provide light (and unintentional pollutant pathway).

2.2. Site 1

The tunnels at Site 1 are situated beneath a main access road to a retail complex. The site consists of two pairs of polymer-concrete tunnels approximately 24 m in length, installed in 2013 (KT 500 climate tunnel with climate slots; KST 700 stilt tunnel sections and KP 1000 entrances, produced by ACO Germany). Traffic mostly comprises cars and buses. The site sits on Sherwood sandstone group bedrock and Alne glaciolacustrine superficial formation of clay and silt deposits [British Geological Survey (BGS), 2017].

2.3. Site 2

The four tunnels at Site 2 are situated beneath a main access road to an industrial park and were installed in 2013. Together, the tunnels and their respective fences cover a 350 m stretch of road, each tunnel approximately 20 m in length. Traffic largely comprises heavy goods vehicles (HGVs) and cars. The site sits on a Lambeth Group bedrock formation of clay, silt and sand and tidal flats from the Paleocene – Eocene epoch, and superficial deposits from the Holocene epoch, consisting of clay and silt [British Geological Survey (BGS), 2017].

2.4. Site 3

The three tunnels at Site 3 are situated beneath a busy arterial ('A') road traversing a designated protected site (Hampton Nature Reserve or Orton Pit SSSI) and were installed in 2006 to reconnect the two areas of habitat which is inhabited by an exceptional *T. cristatus* population of approximately 30,000 individuals; considered to be among the largest populations of the species in Europe (Matos et al., 2017). The tunnels include a very long, 30 m ACO surface tunnel as well two metal sheet and concrete large culverts (5.5 m wide × 2 m high, 40 m long; Matos et al., 2017, 2019; Figure 1). The site is situated on Oxford clay formation of Callovian age – Oxfordian age [British Geological Survey (BGS), 2017].

2.5. Site 4

Site 4 is situated below a residential access road which is estimated to experience a lower rate of daily traffic than Sites 1–3, mostly comprising cars, vans and non-motorised users, e.g., cyclists and pedestrians. The tunnels, three ACOs were installed in 2010 and are each approximately 13 m in length and together cover approximately 100 m stretch of road. The local area was historically industrial, with fired clay brick works and quarries. The site sits on a Passage formation clay bedrock of Arnsbergian sub-age and Langsettian sub-age and Lacustrine silt and clay superficial deposits from the Quaternary period [British Geological Survey (BGS), 2017].

2.6 Reference sites

Reference sites for all four study sites were selected in the local environs to the tunnels in scrub and pond habitat used by amphibians and chosen based on the following criteria: (1) they were at least 200 m from road influences and the tunnel locations (i.e., away from contemporary pollution sources and any drains from roads), and (2) they were located on the same superficial and bedrock geology as the tunnels are situated on at each site, with similar topography (White et al., 2017).

2.7. Field sampling

All four study sites were visited in Autumn (Fall) 2016 prior to application of de-icing salt to obtain a baseline of sediment quality (prior to potential variability associated with de-icing operations in cold months) with six sediment samples of approximately 30 g dry weight taken from the underpass entrances on both sides of the road and from the soil surface at the reference sites, less than 20 cm depth. Samples were collected with a stainless steel trowel, wiped clean between replicates to prevent cross-contamination, and sealed in air-tight plastic sample bags. Smaller quantities of sediment (approximately 20 g dry weight) were also sealed *in-situ* in amber glass jars which were to be taken for external analysis of Total Petroleum Hydrocarbons (TPH) content at Socotec Ltd. (UK). At some of the more recently installed tunnel sites, accumulation of sediment in the tunnels was relatively modest so the number of samples taken per site was inherently volume-limited. As such, the six samples from each tunnel site and associated reference site represented the balance between maximum number of replicate samples and the logistical constraints of sampling where sediment build-up was modest.

2.8. Laboratory analysis

Before analysis, all samples were air-dried in petri dishes in the lab for 5 days, until completely dry. Samples were individually homogenised using a pestle and mortar and separated with a <2 mm sieve. The fine fraction was used for subsequent analyses. For soil pH and electrical conductivity, sub-samples of fine (<2 mm) sediment were mixed with distilled water at a ratio of 5:1 (25 ml distilled water to 5 g dry sediment). Sediment solutions were then mixed using magnetic stirrers for 30 min in line with standard soil paste methods outlined by Johnsson et al. (2005) and ASTM D4972-13 (2013). The pH and electrical conductivity values of the solution were measured using a Myron L Company Ultrameter (6Psi), calibrated on the day of operation.

Organic matter content was measured on 5 g of each sample (after moisture removed at 105oC) at 450°C until a constant weight was achieved. Trace element content on sediments was undertaken on disaggregated fine fraction material which were encapsulated in cassettes topped with 6.0 μ Mylar X-Ray film (TF-160-255). These were analysed with a Thermo Niton XL3t XRF Analyser in soils mode for Ca, Ti, K, Fe, Mn, Zn, Cu, Cr, Zr, and Pb, which are widely used for screening elemental composition of soils and sediments (McComb et al., 2014; Rouillon and Taylor, 2016). Matrix-matched sediment Certified Reference Materials (CRM) were also used throughout this stage of the analysis for quality control and showed consistent accuracy within the range of values apparent in samples (Supplementary Table S1). For analysis of TPH, amber glass jars filled with sediment at source were sent to Socotec Ltd. (Bretby, South Yorkshire, UK) for analysis using gas chromatography with flame ionisation detector (GC-FID) within 2 days of sampling.

2.9. Data analysis

Significant differences in average elemental and major physico-chemical parameter composition between tunnel samples and adjacent reference samples were tested at each site using either Mann Whitney U tests given the small sample sizes (6 samples per site). Principal Component Analysis was used to assess the broader geochemical fingerprint of sediments across the sites and to create a visual representation of the chemical results in relation to their composition and survey site as is often undertaken in such initial studies (Emmerson et al., 1997; Álvarez-Iglesias et al., 2003; Reid and Spencer, 2009). Data were normalised using centre log ratio with values below detection limit recorded at half the detection limit given these accounted for less than 4% of data points (Reimann et al., 2011). Data were analysed in Minitab 17 (for comparisons between tunnels and reference sites) or R (PCA: R Core Team, 2022).

3. Results

3.1. Site 1

Sediment pH was significantly greater in the underpass sediment (Mann Whitney U, $p = <0.001$, median 8.3, range 8.1–8.6) than at the reference site (median = 6.4, range = 6.3–7.1; Figure 2). Neither electrical conductivity or organic content were significantly different between the underpass and reference sites (p values 0.0678 and 0.114 respectively). Most (10 of the 11 tested) parameters showed significant differences in concentration between the underpasses and reference sediments (Supplementary Table S2). Of these, six (Ca, Cu, Pb, Sr, Zn, and TPH) were in elevated concentrations in the underpass sediments (p-values between <0.001 and 0.026; Figure 3). The remaining four (Cr, Fe, K, and Ti) were elevated at the reference site (p-values between <0.001 to 0.049, Figure 3; Supplementary Figure S1). Mn was present in similar concentrations across the two sites (p values 0.743).

3.2. Site 2

Sediment pH was significantly higher in the underpasses compared to the reference site (Mann Whitney U, $p = 0.002$; Figure 2). Electrical conductivity was significantly lower in underpass sediments than reference samples (Mann Whitney U, $p = <0.001$). The median conductivity values of the underpass and reference replicates were 244 and 1154μS cm^{-1}, respectively, (Figure 2). Sediment taken from the underpass had a significantly higher organic content than the reference site (Mann Whitney U, $p = <0.001$; Figure 2). Seven of the 10 tested parameters (Ca, Cu, Mn, Pb, Sr, Zn, and TPH) were in significantly greater concentrations in the underpass than at the reference site (Mann Whitney, p values between <0.001 and 0.0142;

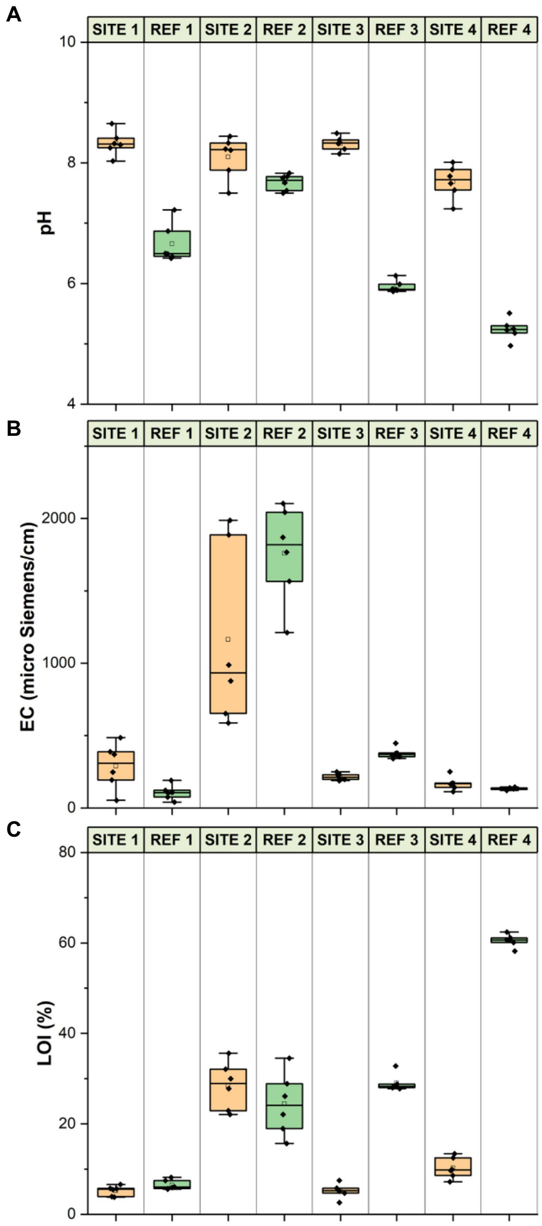

FIGURE 2
Distribution of sediment pH **(A)**, sediment electrical conductivity **(B)** and sediment Loss on Ignition **(C)** between underpass sites (orange) and respective reference sites (green). Data show raw data points as dots, with 95th percentile, interquartile range and median shown by boxplots.

Figure 3; Supplementary Figure S1). Three elements (Cr, K, and Ti) were in greater concentrations at the reference site (*p* values between <0.001 and 0.002). Iron and zirconium were in similar concentrations across the two sample locations and no significant difference was observed (Mann Whitney, $p = 0.301$ and 0.309 respectively).

3.3. Site 3

All three major physicochemical parameters (pH, EC, Loss on Ignition) were significantly different between underpass and reference sites (Mann Whitney, all *p*-values < 0.001). Median pH of the underpass sediment was 8.3, which was much greater than the median pH of the reference sediment which was 5.9 (Figure 2; Supplementary Table 2). Conversely, electrical conductivity and organic content were both significantly greater at the reference site than the underpasses; median conductivity readings were 358.7 and 199.8μS cm^{-1} respectively, and organic matter content 28.8 and 5.7% (Figure 2). Organic content was almost 5× greater at the reference site. Seven of the 11 other tested parameters (Ca, Cu, Mn, Pb, Sr, Zn, and TPH) were in significantly higher concentrations in the underpass sediment than at the reference site (Figure 3; Supplementary Figure S1). Fe, K, and Ti were all in higher concentrations at the reference site (*p* values all <0.001; Figure 3; Supplementary Figure S1). There was no significant difference in chromium concentrations between tunnel and reference sites (Mann Whitney, $p = 0.909$).

3.4. Site 4

Electrical conductivity and pH were both significantly greater in the underpass sediment than the reference sediment (Mann Whitney U test: *p*-values 0.005 and <0.001 respectively). Median pH in the underpasses was relatively neutral at pH 7.68 but was only 5.14 at the reference site (Figure 2). Organic matter content was 7.5 times greater at the reference site than inside the underpasses; 60% compared to 8% (Mann Whitney U-test, $p = <0.001$; Figure 2), which is ascribable to the peat-rich soils in the habitat around the tunnels. Ca, Ti, Zn, and TPH were all found in similar concentrations at the underpass and reference sites and any differences were not statistically significant (*p*-values between 0.852 and 0.6171). Five elements (Cr, Cu, Fe, Mn, and Pb) were abundant in greater concentrations at the reference site (*p*-values between <0.001 and 0.023), and three (K, Sr, and Zr) were in greater concentrations in the underpasses (*p*-values between <0.001 and 0.017: Figure 3; Supplementary Figure S1).

3.5. Multisite comparison

3.5.1. pH and electrical conductivity

When sediment pH values were aggregated across sites the resulting value was significantly greater in the underpass sediment than the respective reference sites at all four study sites (*p* values between <0.001 and 0.01; see Supplementary Figure S2). No significant differences were evident in electrical conductivity or organic content of the sediments when aggregated and analysed together across the four sites (see Supplementary Figure S2).

3.5.2. Major and trace element patterns across sites

Very similar trends were observed between Site 1, Site 2 and Site 3; Ca, Cu, Pb, Zn, and TPH were all in significantly greater concentrations in the underpasses at three sites (*p* values all between <0.001 and 0.026), while K and Ti were all significantly greater at their respective reference sites (see Supplementary Table S2). Site 4 was only similar to the other three sites with concentrations of Sr and Zn, which were both significantly greater in the underpasses. This site departed from the trends of the other three for every other potential variable (Figure 3).

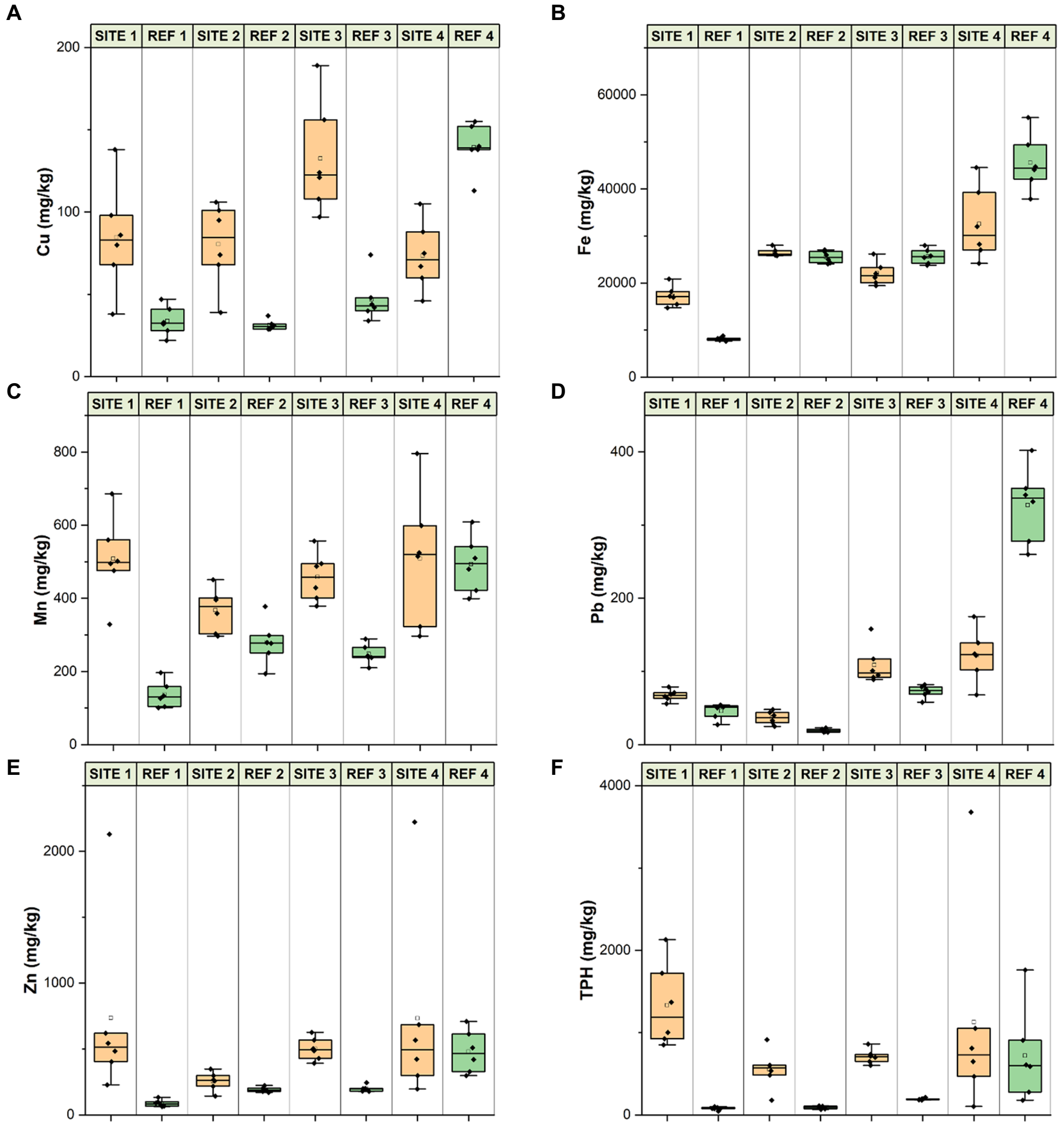

FIGURE 3
Concentrations of a range of potential pollutants between underpass sites (orange) and respective reference sites (green). **(A)** Copper; **(B)** Iron; **(C)** Manganese; **(D)** Lead; **(E)** Zinc; **(F)** Total Petroleum Hydrocarbons. All values in mg/kg dry weight sediment. Data show raw data points as dots, with 95th percentile, interquartile range and median shown by boxplots.

The Principal Component Analysis performed on the underpass sediment across all four sites and their respective reference sites shows where sites were similar (sites plot close together) or dissimilar (sites plot far apart) in chemical composition (Figure 4). Three significant components accounted for more than 10% of the variance individually (PCA 1 = 0.33.4, PCA 2 = 0.265, PCA 3 = 0.153). Cumulative of the 3 PCAs = 0.739 (75.2%). There is significant clustering of underpass sites in the centre to upper right of Figure 4 characterised by elevated Ca, Sr, TPH, Mn, Cu, and Zn, as well as elevated pH compared to most reference sites. The reference sites typically plot in the lower left (Sites 1–3) and typically poor in concentrations of those parameters, and lower in pH, but with relative enrichment of K, Ti, and organic matter (LOI). The Site 4 reference samples are a clear outlier from both underpass sites and other reference sites, being enriched in organic matter (LOI), Pb, Fe, and Ti in particular (Figure 4).

4. Discussion

The data presented provide the first multisite assessment of potential pollutant build up in sediments of amphibian mitigation underpasses in the UK. There was significant elevation of Zn, Cu, Pb, and Total Petroleum Hydrocarbons (TPH) at three of the four study sites above adjacent reference site values. A comparison with regulatory sediment quality guidance (see Table 1 for definitions) shows concentrations of Zn, Cu, and Pb all exceed the lower screening values with Zn concentrations above the more precautionary Probable Effects Level (PEL) threshold at three sites. Even at Site 4, where tunnel sediment metal concentrations (Cu, Pb, and Zn) were either significantly lower or showed no difference from reference site values, the absolute metal concentrations are still in a range considered potentially problematic according to sediment quality screening

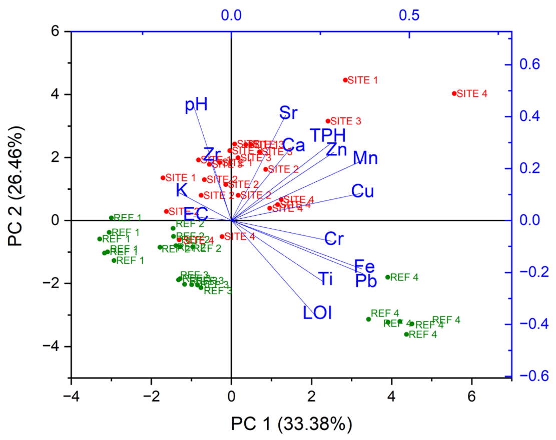

FIGURE 4
Principal Component Analysis of sediment chemical composition across the four study sites. Individual samples shown as points (red points: underpasses; green points: reference sites). Eigenvectors in blue show key characteristics of sample stations and inter-relationships between variables (where they plot in a similar direction and extent, variables are positively correlated). Based on scree analysis, there are three significant components accounting for a cumulative variance of 75.2% in the data.

TABLE 1 Guidance values for good soil and sediment quality, including the UK Environment Agency's non-statutory Soil Screening Values (SSVs: Environment Agency, 2017), the United States Ecological Soil Screening Levels (ECO-SSLs), and Threshold Effects Levels (TEL) and Probable Effects Levels (PEL – 50% adverse effects occur above this) taken from NOAA Buchman Screening Quick Reference Tables (2008).

	EA SSVs	EPA ECO-SSLs	TEL	PEL
Cu	57.8	5.4	35.7	197
Pb	167.9	0.05	35	91.3
Zn	116.1	--	123	315

None of these limits have been set specifically for amphibians but rather for general soil quality. The EPA ECO-SSL values (United States Environmental Protection Agency, 2007) are based on bioassays of water vole and shrew (mammals), which were the most sensitive species in the guidance. For the purpose of this study, these values are taken as an absolute maximum, under the knowledge that amphibians are more sensitive than mammals. All values in mg/kg.

criteria (Environment Agency, 2004; Buchmann, 2008). The unusually high metal concentrations in reference soils at Site 4 were despite the sample locations showing no link with contemporary or historical sources of water pollution. However, the lowland raised bog site lies downwind of what was historically a major centre for coal mining and iron and steel production (Riley et al., 2020) and as such the soils and sediments may reflect regional metal-enrichment from historical atmospheric deposition. Crucially, the absolute values of potential pollutants in tunnels are similar across all sites suggesting similar pollution sources and pathways between road surfaces and underpasses.

Zn concentrations ranged between 258 and 567 mg/kg in the underpasses (Figure 3), which exceeded the Environment Agency Soil Screening Values (EA SSVs: Environment Agency, 2017) and Threshold Effects Levels (TEL: Environment Agency, 2004) at all sites. Site 1, 2, and 4, all exceeded the PEL of 315 mg/kg, though Site 3 did not. Zinc has been singled out as a particularly common hazard occurring in highways dusts in high concentrations (Świetlik et al., 2015) and is associated with tyre wear, corrosion of road barriers and brake disc wear (Blok, 2005). The data presented here suggest modest to high Zn concentrations are ubiquitous in amphibian underpasses at the sites sampled in concentrations of potential ecological significance.

Mean underpass concentrations of Cu across the four study sites ranged between 67 and 124 mg/kg (Figure 3), which were all greater than the lower soil guidance values at all study sites, but lower than the Probable Effects Level (PEL). The only standard that no sites were greater than was the more precautionary PEL (197 mg/kg), which Site 2 was closest to exceeding (average of 124 mg/kg). Cu concentrations in road dust have been shown to be more variable than Zn, being driven largely by wear and tear from braking, with higher concentrations around road junctions and intersections (Bäckström et al., 2004).

Mean concentrations of Pb across the four underpass sites (range 40–124 mg/kg; Figure 3) exceeded the US ECO-SSL (United States Environmental Protection Agency, 2007) and TEL at all sites, and at sites 2 and 4 concentrations additionally exceeded the PEL. Since the phasing out of lead additives in automobile fuel, Pb release associated with vehicles has fallen dramatically (Hwang et al., 2016). However, residual sources of Pb can be associated with tyre and brake wear as well as the loss and subsequent pulverisation of wheel weights (Świetlik et al., 2015).

Manganese (Mn) is present in underpass sediments in concentrations that are significantly elevated above reference sites at three of the four sites (Figure 2). Although there remains uncertainty about direct sources of Mn in highway dusts, Mn is used in anti-knocking fuel additives and can be released from brake wear (Fujiwara et al., 2011), and has been found in elevated concentrations in road dust (Świetlik et al., 2013). There are no formal UK soil or sediment screening guidance to compare absolute Mn values, but along with Ca, Cu and Zn, Mn is a key indicator of underpass sediment composition (Figure 4).

There are no formal standards or screening values for TPH values, but the data show significant elevations of TPH in all underpass sediments compared to reference conditions (Figure 3). The TPH method does not categorically show the hydrocarbons present are of petroleum origin (as analysis is subject to interference from organic matter and chlorinated solvents: Villalobos et al., 2008), but the generally lower organic matter concentrations in the underpass sediments are likely to limit such interference compared to reference sites (Supplementary Figure S2). As such the data suggest a potential pathway of exposure from petroleum hydrocarbons spilled on the road surface to the amphibian underpasses and should be the focus of more detailed analyses and attention.

The broader matrix of the tunnel sediments is generally consistent across the sites, being more mineral-rich (notably Ca and Fe) and displaying lower concentrations and clay-forming elements (e.g., K and Ti) than adjacent reference sites which typically have clay-rich superficial deposits (Figures 3, 4). Only at Site 3, where the larger culverts had a local soil substrate imported does the broader matrix reflect reference conditions. The generally higher inorganic content of the underpass sediments is reflected in the circum-neutral to alkaline pH reflecting the mineral-rich highways dusts as well as the weathering of tunnel construction materials and road surfaces (Gomes et al., 2016; White et al., 2017). Under this pH range, the mobility of most metals should be relatively restricted (Langmuir, 1997), which is tentatively positive. Furthermore, studies elsewhere of road dust metal mobility typically demonstrate metals present in hard-to-leach phases of low mobility (Świetlik et al., 2015). None of the sediments displayed highly alkaline conditions that have been previously documented in the drainage waters at one of the study sites (Site 1) and associated with leaching from cement in the tunnel walls (White et al., 2017).

Highway de-icing operations are a potential key threat to amphibians and widely documented as a pollutant pressure in many studies (e.g., Karraker and Ruthig, 2009; Denoël et al., 2010). The underpass sediment electrical conductivity (EC) values collected here did not show consistent elevation above reference sites (only Site 4 did: Figure 2), suggesting the accumulation and release of soluble salts from de-icing operations are of limited importance at the time of sampling (Autumn/Fall), but seasonal sampling suggests these underpass sediment EC can be variable during the course of the year (White, 2019) and should be a focus of future research.

These data provide a first multisite assessment of potentially common highways-derived pollutants in amphibian mitigation tunnels and highlight a pathway of potential movement of contaminants from road surfaces to tunnels. However, further research is needed to assess the subsequent risk associated with these pollutants (as well as other highways-derived pollutants such as microplastics: Kole et al., 2017) which should focus on potential bioavailability and likelihood of remobilisation into the water column during flood events (e.g., into adjacent ponds used by amphibians). More refined analyses of sediments to assess specific organic pollutants present and analyses that establish whether tunnel sediments reflect weathering products of the polymer-concrete tunnels themselves (e.g., through sediment carbonate analysis: e.g. Heiri et al., 2001) would be beneficial. Furthermore, seasonal assessment of changes in both sediment and water quality would be beneficial to understand and identify any short-term deterioration of water quality in receiving tunnels and adjacent habitat (e.g., rainfall events after de-icing salt application: Gallagher et al., 2011) and how these compare with critical times of year for amphibian movement. A degree of caution is required in the interpretation of these results as the current study was limited by funding for chemical analyses and aimed to cover a wide range of potential pollutants given that it is an initial investigation. Future studies should focus on specific elements included here and extend the surveys to more sites and multiple seasons, which would also allow a more robust analysis.

5. Management implications

It is clear from the literature on underpass effectiveness that although knowledge gaps remain for numerous species and types of mitigation designs, this form of mitigation can be successful at reducing habitat fragmentation and direct mortality *via* collisions with vehicles for amphibians (Schmidt and Zumbach, 2008; Hamer et al., 2015; Helldin and Petrovan, 2019; Jarvis et al., 2019; Schmidt et al., 2020). However, pollution remains very poorly quantified and the findings of this and previous studies (White et al., 2017) caution for the need for further consideration of highway-derived pollutants in the designing, installation and maintenance of such underpasses. The design of systems should endeavour to break any potential pathway between surface runoff on highways and the tunnels, for example by having road drains placed immediately up-gradient from tunnel vents and indeed and this is sometimes considered for ACO-type tunnels, which are positioned so that rainfall runoff is not directed towards the open grid at the top instead of normal water drainage. However, such aspects should be better clarified in technical guidance documents and then followed up during post-implementation monitoring. Importantly, it is relevant that while most of the underpasses we included were >20 m long, the road surface itself was much smaller and sometimes only represented a third of the tunnel length, with the rest taken by pedestrian access areas, cycle tracks and raised grass verges. This indicates that there might be simple compromises where the open grid tunnel sections would only be installed in the non-traffic areas and the part actually under the road could be kept without such open grids, thus reducing the opportunity for road pollutants to enter and accumulate in the tunnel sediment. However, any such design modifications should be implemented cautiously and include careful testing given that they might negatively impact on the acceptance of the structure for amphibians, including the great crested newt, which already show a propensity for U-turns and abandoning the tunnel crossing (Jarvis et al., 2019; Matos et al., 2019).

In addition, the variety of mitigation solutions used mean there is a need to target additional sites of different construction type for specific pollutant monitoring, both in the underpass and alongside the barrier fencing in order to create a clearer image on this topic. There is also a need to include other types of pollution and especially microplastic from tyre wear, which has been shown to be a significant issue in road dust (Leads and Weinstein, 2019). The impact of microplastics on amphibians of different life stages are very poorly understood but might be significant (Boyero et al., 2020; da Costa Araújo et al., 2020).

For existing tunnels and underpasses where highways-derived pollution might be an obvious problem (e.g., due to heavy vehicle or high volume traffic), potentially the most effective solution for maintenance may be to periodically jet-wash the inside of the underpasses to clear them of any contaminants from the road surface. Whilst sediment accumulation is attractive to amphibians and may increase usage rates compared to bare concrete surface inside the underpass (Lesbarrères et al., 2004), it is counter-productive if that sediment contains potentially harmful elements for amphibians. Tunnel jet-washing is already common practice in some European countries, e.g., Germany and is typically carried out at the end of winter, in order to remove potential road salt but also to clear the underpass from accumulated leaf litter which can be a significant issue and can prevent usage by amphibians by blocking the underpass. Issues of longer-term maintenance responsibility however need to be considered and clarified during planning (i.e., would the developer be responsible for long-term management of the underpasses or would local councils or even volunteers from conservation organisations). While the costs of such maintenance are usually low, in practice this can be a serious issue if there is no specific agreement in place and thus the entire mitigation structure might in fact be abandoned after the required monitoring period which is typically 5 years for protected species such as *T. cristatus* in the UK (Matos et al., 2017).

6. Conclusion

From the results of this study, it could be concluded that highway-derived pollutants are accumulating in road underpasses installed primarily for *Triturus cristatus*. Four potential contaminants (Cu, Zn, Pb, and TPH) were accumulating in the underpasses at three out of four study sites in levels above potential soil and sediment quality thresholds, suggesting that contamination is not an isolated occurrence at one development site yet the potential impacts of this remain unknown. The contaminated sediments could provide a secondary source of water pollution after rainfall events that may be of significance to amphibians using the tunnels. Further research is recommended, to include more refined analyses of contaminant form, to incorporate investigations of further sites, and consider bioavailabilty of potential contaminants. Ecotoxicological studies of *T. cristatus* and other amphibians utilising the tunnels to determine whether contamination is affecting their overall health and population size would also be desirable.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KW collected and analysed the samples. KW, SP, and WM contributed to the experiment design, interpretation, and manuscript writing. All authors contributed to the article and approved the submitted version.

Funding

The researchers are grateful for funding at Froglife Trust by the Esmée Fairbairn Foundation under grant NR 14-0534.

Acknowledgments

We thank the three reviewers for their comments and suggestions on the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1133253/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 17 April 2023
DOI 10.3389/fevo.2023.1008462

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 31 July 2022
ACCEPTED 22 March 2023
PUBLISHED 17 April 2023

CITATION
Harman KE, Omlor K and Popescu V (2023)
Evaluating fence-end treatments for migratory amphibians.
Front. Ecol. Evol. 11:1008462.
doi: 10.3389/fevo.2023.1008462

Evaluating fence-end treatments for migratory amphibians

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Migratory amphibians require movements to complete their biphasic life cycle, often across altered landscapes fragmented by roadways, which can have severe consequences on their populations. To manage this threat, transportation agencies have begun to implement exclusion fencing to separate natural areas from the roadway to prevent wildlife-vehicle collisions. Although fences are an effective conservation tool, the tendency of animals to access the road by circumventing the fence ends, known as the fence-end effect, threatens to jeopardize management efforts to reduce road associated mortality. One strategy to lessen the impacts of the fence-end effect is to construct fence-end treatments to block amphibian movement and guide the animals to safe crossing locations. By using experimental fence arenas, we examined how nine amphibian species responded to two alternative fence-end structures: horizontal v-shape and perpendicular fence-end treatments. Using a generalized linear model framework, we found both fence-end treatments to be an effective strategy to reduce the impacts of the fence-end effect, with our predictor variable, fence-end treatment, explaining most of the variation in amphibian response. Structure effectiveness also started to improve by 20% with each 7°C increase in temperature, however, this was not significant. Despite these promising findings, we also found for each additional 312 s an amphibians spent attempting to navigate around the experimental fence resulted in a 25% decline in structure effectiveness, suggesting longer fences are not an adequate protection measure to combat the fence-end effect for amphibians. In addition, *Anaxyrus americanus* was not found to differ in their response, performing equally well to both experimental fence-end treatments. In contrast, *Rana* spp., *Pseudacris crucifer*, and *Notopthalmus viridescens* showed a greater response to the horizontal v-shape fence-end treatment compared to the perpendicular fence-end treatment. Variation in response for *Ambystoma* spp. could not be detected due to a small sample size; however, no individuals responded positively to the perpendicular fence-end treatment. Guidelines for amphibian fences should continue to incorporate fence-end treatments into the design and implementation to mitigate for the fence-end effect, and preferably angle the fence-ends inward in the horizontal v-shape pattern with the fence ends diagonal to the road for migratory amphibians.

KEYWORDS

road mortality, fence-end effect, amphibian tunnel, road mitigation, road ecology

1. Introduction

Roads that fragment habitats and are constructed within 2 km of a wetland are a continuous source of additive mortality that results in the loss of hundreds of thousands of pond-breeding amphibians each year (Fahrig et al., 1995; Ashley and Robinson, 1996; Forman and Alexander, 1998; Forman et al., 2003; Glista et al., 2008; Gryz and Krauze, 2008; Fahrig and Rytwinski, 2009; Andrews et al., 2015). Unlike species that adapt their behavior to cope with road threats (Andrews and Gibbons, 2005), amphibians do not demonstrate road avoidance behaviors and will readily attempt to cross a road that bisects their migratory pathway (Beebee, 2013). Additionally, amphibians are generally small-bodied animals with slow movement patterns that commonly rely on cryptic coloration and immobility to respond to external threats, which prolongs the time spent crossing a road and increases the likelihood of being struck (Hels and Buchwald, 2001; Mazerolle et al., 2005; Andrews et al., 2006; Rytwinski and Fahrig, 2012). For these reasons, migratory amphibians frequently experience higher rates of road mortality compared to other vertebrates (Fahrig and Rytwinski, 2009). Therefore, preventing migratory amphibians from accessing the roadway during seasonal migration events is critical for the long-term sustainability of their populations (Jaeger and Fahrig, 2004; Rytwinski and Fahrig, 2012).

When the primary management objective is to reduce occurrences of road mortality caused by wildlife-vehicle collisions, installing an artificial barrier system to partition natural areas from traffic, called wildlife exclusion or barrier fences (hereafter, *fences*), is encouraged (Forman et al., 2003; van der Ree et al., 2015). Previous studies assessing changes in roadkill frequencies and their distribution after implementation of a fence found significant declines (86—98%) in the number of amphibian carcasses detected within the fence boundaries (Rytwinski et al., 2016). In addition, fences have also been shown to increase wildlife movement by directing animals to safe crossing locations, typically artificial structures built either above (bridges) or below (tunnels) the road called eco-passages (Forman et al., 2003; Andrews et al., 2015; van der Ree et al., 2015). Thus, if maintained, fences can be a long-lasting conservation tool for mitigating the detrimental effects of roads, particularly if paired with eco-passages (Dodd et al., 2004; Aresco, 2005). Although many studies have demonstrated fences improve use of eco-passages and effectively reduce the frequency of road mortality detected within the boundary walls, an increased concentration of road mortality occurring at the fence ends suggest management efforts are compromised (Dodd et al., 2004; Aresco, 2005; Rytwinski et al., 2016; Markle et al., 2017), an unintended consequence known as the "fence-end effect" (Huijser et al., 2016; Plante et al., 2018; Spanowicz et al., 2020).

To combat the fence-end effect, management guidelines propose installing a barrier system to block animals from circumventing the fence ends, called fence-end treatments (Huijser et al., 2016). For smaller, less mobile animals such as amphibians, two different types of fence-end treatments have been deployed: (1) bend the fence ends inward *perpendicular* to the road at a 90° angle or (2) bend the fence ends further inward, creating a 45° angle, in a *horizontal v-shape* (Huijser et al., 2015; Gunson et al., 2016). Animal movements can then either be guided back to the landscape from which they emerged or redirected back to eco-passages built under the road for amphibians (Dodd et al., 2004; Aresco, 2005; Huijser et al., 2016). Assessments to date suggest structure design for herpetofauna should be guided by the habitat requirements an animal needs to survive and reproduce (e.g., whether the animal can be guided back to the landscape from which they emerged or if they need to be redirected back to eco-passages to cross the road; Dodd et al., 2004; Aresco, 2005; Huijser et al., 2016). For migratory amphibians, if the terrestrial and wetland habitats are separated by a road, fence-end treatments should encourage them to move in the opposite direction of the structure toward an eco-passage. Thus, given the substantial financial commitment to implement road mitigation structures (Forman, 2000), more research consideration is warranted to determine if migratory amphibians respond to the current fence-end treatment recommendations to maximize the conservation outcome to preserve their populations.

The purpose of this study is to improve fence design practices by evaluating fence-end treatment effectiveness for nine commonly occurring migratory amphibians, for which high rates of road mortality endangers long-term persistence of these populations (Fahrig and Rytwinski, 2009; Hopkins et al., 2019). Specifically, our study objectives are (1) to determine if fence-end treatments mitigate for the fence-end effect, and (2) to compare the effectiveness of the perpendicular and horizontal v-shape fence-end treatments for migratory amphibians. To accomplish our objectives, we created experimental arenas with an attached fence-end treatment. As a result, we were able to observe if movement of migrating breeding adults were affected by the presence of an experimental fence-end treatment when they encountered a replica fence structure. If fence-end treatments effectively mitigated for the fence-end effect, we hypothesized more amphibians would turn away from the fence-end treatment and move around the fence end that remained unobstructed (i.e., toward the hypothetical eco-passage entrance). As a result, we expected less amphibians would circumvent the fence-end treatment side, which would suggest amphibian response to a fence-end treatment is deliberate. Thus, fence-end treatments would effectively lessen the intensity of the fence-end effect by hindering movement onto the road and guiding amphibian movements back to safer locations. In addition, previous studies have shown species-specific responses to different structural attributes of crossing structures (Woltz et al., 2008; Hopkins et al., 2019). Because amphibians are a diverse clade displaying distinct morphological and behavioral differences (Wells, 2007; Pfingsten et al., 2013) with varying degrees of vagility (Hels and Buchwald, 2001), we predicted amphibian species would display a species-specific response, showing a greater preference for one of the fence-end treatments.

2. Materials and methods

2.1. Experimental fence arenas

We constructed four experimental arenas to test amphibian response to either the perpendicular fence-end treatment or the horizontal v-shape fence-end treatment. The perpendicular fence-end treatment was created by bending the fence ends inward to create a 90° angle between the treatment and the fence (**Figure 1**)

FIGURE 1
Experimental fence arenas used to evaluate the perpendicular fence-end treatment effectiveness in Athens County, Ohio, 2020. The arenas measured 58/81 cm × 1.2 m × 2.4 m with a 1 m experimental perpendicular fence-end treatment with a 90° angle attached to one side (showing attached to the right side). Amphibians were placed on a designated spot (black dot) in the middle of the experimental setup and positioned 15 cm from the fence.

FIGURE 2
Experimental fence arenas used to evaluate the horizontal v-shape fence-end treatment effectiveness in Athens County, Ohio, 2020. The arenas measured 58/81 cm × 1.2 m × 2.4 m with a 1 m experimental horizontal v-shape fence-end treatment with a 45° angle attached to one side (showing attached to the left side). Amphibians were placed on a designated spot (black dot) in the middle of the experimental setup and positioned 15 cm from the fence.

compared to the horizontal v-shape fence-end treatment that was bent further inward to create a sharper 45° angle between the treatment and the fence (**Figure 2**). These arenas were then placed within 3 m of an established breeding wetland along State Route 78 in Athens County, Ohio (39.449297 and −82.198720). We then positioned the arenas so that the fence-end treatment faced toward the terrestrial habitat to intercept reproductive adults as they migrated to the wetlands. Under this setup, we were able to observe how breeding adults navigated around a mitigation fence under natural conditions. Fence arenas were constructed from a 1 m tall silt fencing material held up with wood posts (Willson and Gibbons, 2010). The fence segments were 2 m long with a 0.5 m experimental fence-end treatment attached to either the left or right end. We then lined the top of each fence with multiple 142 g weights to create an overhanging lip. The fence arenas were set flush with an untreated plywood board (58/81 cm × 1.2 m × 2.4 m) to provide a level substrate with no obstructions to movement. Arenas were weathered onsite to create a naturally moist substrate for 1 month prior to the start of the trials.

2.2. Behavioral trials

Amphibians were collected from pitfall traps located at the end of an existing mitigation fence and hand collected from the road within 0.4 km from the experimental site (the total length of the migration corridor based on a multi-year road mortality survey; see Hopkins et al., 2019). The trials lasted seven and half weeks in the spring (4 February 2020—31 March 2020) and two and half weeks in the fall (11 October 2020—29 October 2020) for a total of 29 test nights. We processed all amphibians and recorded species, weight (g), snout-vent length (mm), and sex (Dodd, 2010). We tested nine amphibian species that commonly occur in the area: American Toad (*Anaxyrus americanus*), Spring Peeper (*Pseudacris crucifer*), Green Frog (*Rana clamitans*), Leopard Frog (*R. pipiens*), Pickerel Frog (*R. palustris*), Wood Frog (*R. sylvatica*), Red-spotted Newt (*Notopthalmus viridescens*), Jefferson Salamander (*Ambystoma jeffersonianum*), and the Spotted Salamander (*A. maculatum*). Anurans and *Ambystoma* spp. were all reproductive adults, whereas *N. viridescens* complete maturation after returning to natal ponds so were recorded as non-reproductive subadults (Petranka, 1998). Experimental animals were held individually in a 19 L plastic bucket prior to testing (Woltz et al., 2008). We were unable to avoid direct contact with animals prior to the trial because some amphibian species can climb the bucket wall. Therefore, to maintain consistency across all species, we placed all animals by hand on a designated spot in the middle of the experimental setup positioned 15 cm from the fence (Woltz et al., 2008). Prior to sunset, we randomized the order of the arenas to control for potential orientation cues influenced by calling males in the adjacent ponds or olfactory scent trails of other migratory individuals (Sinsch, 1991). All trials were conducted on rainy nights, starting at least 30 min after sunset, and typically concluded before midnight, except for a single explosive migration event that concluded at 3:00 a.m. After each trial, individuals were released into the adjacent wetland within 3 h of capture.

Test animals were observed as they traversed the experimental fence and the trial concluded when the test animal walked around one of the fence ends (treated vs. non-treated side). We recorded whether the animal responded to the treated side with the attached experimental fence-end treatment as success or fail. A “successful response” was recorded when an animal moved around the non-treated fence end either directly or after the animal approached the fence-end treatment but returned toward the non-treated side. A “fail response” was recorded when an animal escaped the fence arena by circumventing the experimental fence-end treatment. Anurans were given 20 min to make a fence-end choice (movement around one of the fence ends) and we extended the length of trial to 25 min for caudate to accommodate for their slower movements. If an animal remained on the board after the given time (i.e., trapped at the fence) or walked off the back of the board (i.e., abandoned the fence), a “no choice” was recorded and these animals were removed from analysis (Woltz et al., 2008). Amphibians were randomly assigned to one of the arenas, and only tested once and independent to other test individuals. Weather variables were taken from a weather station (KOHBUCHT1; 39.46°N, 82.189°W) located within 0.5 m from the field site.

2.3. Data analysis

Variation in our response variable, fence-end choice, was examined using a generalized linear model with a binomial distribution of errors in R (R Core Team, 2016). Fence-end choice was coded as 1 (success) or 0 (fail). Continuous variables were checked for correlation *via* Pearson's correlation coefficient, *r*, to test the strength of association between -1 and 1. If sets of continuous variables were highly correlated ($>|0.7|$) we removed one variable of the set from analysis. Wind speed and wind gust were the only variables found to be highly correlated ($r = 0.99$). We then removed wind gust from analysis. Our final set of continuous variables were then standardized. We then used Akaike's Information Criterion (AICc), corrected for small sample sizes, in a model selection framework to identify important factors influencing response to an experimental fence-end treatment for amphibians. Models incorporated combinations of the following parameters: (1) environmental variables, (2) experimental variables, and (3) individual variables (**Table 1**). Environmental variables included: temperature (°C) at the time of the experiment and precipitation rate (in/hr) because these variables have been positively correlated with amphibian activity (Gibbons and Bennett, 1974; Sexton et al., 1990). We also included how long after sunset the trial started, recorded as time since sunset, (the difference in seconds between the time the trial started from the time of last light; Hels and Buchwald, 2001) and date of the trial (day of the year coded as Julian day; Mazerolle, 2001). Experimental variables included treatment type (horizontal v-shape or perpendicular) and length of trial (the total time in seconds it took to move around one of the fence ends). Individual variables included sex, genus, and fence experience (coded as *experienced* for test animals collected from pitfall traps at the mitigation fence or *virgin* if the test animals were collected from the road). We then used a model selection procedure to determine the best supported models with the lowest AICc score using the package *MuMin* in R. When a top model was identified, we calculated the odds ratio expressing relative contribution of various predictor variables for explaining the likelihood of fence-end choice (R Core Team, 2016; Barton, 2020; Pinheiro et al., 2020).

Lastly, we used a goodness of fit *G*-test with a Yates correction to evaluate species-specific response to the perpendicular and horizontal v-shape experimental fence-end treatments. We observed amphibians were 50% likely to move either toward or away from the fence-end treatment upon their initial interactions with the fence. Therefore, we assumed a 50:50 probability of randomly circumventing either the treated side with the experimental fence-end treatment or the untreated side that remained unobstructed if the fence-end treatment had no effect on individual response. We grouped species into genera because of the small sample size of the *Ambystoma* spp. and *Rana* spp. Statistical significance was assessed at $\alpha = 0.05$ *via* a two-tailed test.

3. Results

We collected 302 individuals during the spring migration and 34 individuals (all *N. viridescens*) during the fall migration: *A. americanus*, $n = 149$; *P. crucifer*, $n = 57$; *Rana* spp., $n = 39$; *Ambystoma* spp., $n = 7$; *N. viridescens*, $n = 84$ (**Table 2**). Most amphibians made a fence-end choice: *Ambystoma* spp. made a fence-end choice most frequently (100%), followed by the *A. americanus* (73%), *P. crucifer* (65%), *N. viridescens* (61%), and *Rana* spp. (56%). Of those that made a fence-end choice, between 72 and 75% successfully responded to the fence-end treatment for all genera except *Ambystoma* spp. where only 42% responded positively (**Table 2**). Of the individuals that did not make a fence-end choice, *P. crucifer* and *A. americanus* were the least likely to abandon the fence arena (9 and 20 %, respectively), whereas we observed 57 % of *Rana* spp. and 61% of *N. viridescens* demonstrate arena abandonment (**Table 2**). *Rana* spp. were the most likely to remain on the board for the entire length of the trial (41%) compared to *P. crucifer* (26%), *N. viridescens* (18%), and *A. americanus* (7%). *N. viridescens* were on average ($\bar{x} \pm \sigma$) the slowest to complete the fence arenas (400.1 ± 347.0 s), while *Ambystoma* spp. were on average ($\bar{x} \pm \sigma$) the fastest (233.1 ± 158.1 s), followed by *P. crucifer* (353.7 332.2 s), *A. americanus* (326.2 ± 275.0 s), and *Rana* spp. (323.2 ± 369.4 s).

Fence-end treatment and length of trial were the top factors explaining amphibian response to a fence-end treatment in our dataset (**Table 3**). Both the horizontal v-shape and perpendicular fence-end treatments were positively associated with success and were efficient at directing amphibians toward the non-treated side of the fence (horizontal v-shape, odds = 4.87, 95% CI [3.01, 8.34], $p < 0.0001$; perpendicular, odds = 2.32, 95% CI [1.56, 3.52], $p < 0.0001$). Conversely, length of trial was negatively correlated with success; however, this was not significant (odds ratio = 0.75, 95% CI [0.56, 1.02], $p = 0.06$). Temperature at the time of the trial was also found to be a potentially important factor explaining treatment response, but the model that included this variable was slightly greater than 2.0 AIC units from the top model (**Table 3**). However, temperature also showed a positive trend associated with success, though the relationship was also not significant (odds ratio = 1.20, 95% CI [0.88, 1.63], $p = 0.24$). Our data suggests that for each additional standard deviation in time spent navigating (312 s) the fence arena, there was a 25% decline in the likelihood to respond successfully to a fence-end treatment. However, in contrast, for each additional standard deviation increase in temperature (7°C), there was a corresponding 20% increase in the likelihood of successfully responding to the experimental fence-end treatment.

In testing for species-specific response to one of the fence-end treatments (**Figure 3**), *A. americanus* was the only species to demonstrate a successful response to both treatment types (horizontal v-shape, $G = 10.12$, $p < 0.05$; perpendicular, $G = 17.29$, $p < 0.05$), whereas *Rana* spp. and *P. crucifer* showed only a successful response for the horizontal v-shape fence-end treatment (*Rana* spp.: $G = 5.42$, $p < 0.05$; *P. crucifer*: $G = 15.84$, $p < 0.001$), but not the perpendicular fence-end treatment (*Rana* spp.: $G = 0.76$, $p = 0.38$; *P. crucifer*: $G = 0.50$, $p = 0.48$). Similarly, *N. viridescens* also showed a successful response for the horizontal v-shape treatment ($G = 17.32$, $p < 0.001$) but not the perpendicular fence-end treatment ($G = 2.41$, $p = 0.12$). Due to small sample size,

TABLE 1 Variables considered in the analysis for evaluating amphibian response to fence-end treatments in the spring and fall 2020.

Variable name	Definition/Explanation	Environmental, experimental, or individual variable	Range of values and units	Related to hypothesis	Variable type
Temperature	Hourly temperature measured from the nearest weather station	Environmental	2.4–9.8 °C	1	Fixed
Precipitation rate	Amount of water deposited during a specific length of time measured from the nearest weather station	Environmental	0–0.31 in/hr	1	Fixed
Time since sunset	The difference in time between the time the trial began from the time of the last light of sunset	Environmental	18–385 s	1	Fixed
Date	The day of the trail	Environmental	56–302 (Julian day)	1	Fixed
Treatment type	The type of fence-end end treatment attached to the experimental arena	Experimental	Horizontal v-shape or perpendicular	1, 2	Fixed
Length of trial	The total time it took for an individual to move around one of the fence ends	Experimental	3–1407 s	1	Random
Fence experience	Individuals that were captured from pitfall traps or collected along the road	Individual	Experienced or virgin	1	Random
Sex	The sex of each test individual	Individual	Male, female, or, subadult	1, 2	Random
Genus	The species of each test individual grouped into their taxonomic rank	Individual	Anaxyrus, Pseudacris, Rana, Ambystoma, Notopthalmus	1, 2	Random

TABLE 2 Summary data for the experimental fence arenas including mean and standard deviation ($\bar{x} \pm \sigma$) for weight (W), snout-vent length (SVL), and the number of individuals (*n*) that made a FEC, fence-end choice; AB, abandoned the fence arena, or TR, remained trapped on the board after 20 min for anurans or 25 min for caudates for males (M) and females (F).

	Anaxyrus (n = 149)		Pseudacris (n = 57)		Rana spp. (n = 39)		Ambystoma spp. (n = 7)		Notopthalm (n = 84)
	M	F	M	F	M	F	M	F	
W (g)	22.0 ± 6.9	49.1 ± 23.0	1.7 ± 0.6	2.4 ± 0.6	31.7 ± 17.6	31.7 ± 17.6	17.4 ± 4.5	17.7 ± 4.6	2.3 ± 1.0
SVL (mm)	52.0 ± 8.8	63.5 ± 6.0	25.9 ± 2.9	29.2 ± 2.0	53.3 ± 8.9	79.4 ± 44.7	79.5 ± 5.3	85.0 ± 6.2	39.8 ± 5.3
FEC (n)	87	22	23	14	12	10	4	3	51
Direct (n)	60	15	16	7	9	5	1	1	37
Return (n)	6	1	3	3	0	3	0	0	3
AB (n)	26	4	2	3	1	0	0	0	18
TR (n)	10	0	10	5	14	2	0	0	15
Total (n)	123	26	35	22	27	12	4	3	84

Notopthalmus were all non-reproductive subadults and were therefore not sexed. Of the individuals that made a fence-end choice, the number of individuals (*n*) that moved directly to the non-treated side (Direct) or moved to the treated side, encountered the fence-end treatment, and then returned to the non-treated side (Return) is also reported.

no significant difference in a successful response was detected for *Ambystoma* spp. for either treatment type (horizontal v-shape: $G = 1.46$, $p = 0.23$; perpendicular: $G = 2.53$, $p = 0.11$).

4. Discussion

Our analysis indicates fence-end treatments are an effective conservation tool to mitigate for the fence-end effect for migratory amphibians; treatment ranked in our top three models, carrying a cumulative AICc weight of 0.79. Knowledge of fence-end treatment effectiveness is limited, as few studies have evaluated before and after measures of roadkill frequencies after installing a mitigation fence with fence-end treatments (see review by Rytwinski et al., 2016), however, those that have assessed effectiveness of mitigation fences with fence-end treatments reported similar benefits for herpetofauna. For example, Aresco (2005) found mitigation fences with perpendicular fence-end treatments protected an estimated 98% of aquatic turtles and 73% of amphibians, excluding hylids, from accessing the highway. However, these structures extended 50–150 m and followed the edge of the lake; thereby, created a "pseudo-pen" that primarily functioned to guide turtles and other herpetofauna back to the aquatic habitat from which they emerged. In another study conducted by Huijser et al. (2016), the authors found similar results for ungulates: longer fences greater than 5 km resulted in at least 80% reduction in wildlife-vehicle collisions at the fence ends. Elongated fence-end treatments like these structures can be problematic when the road bisects migration corridors and movement across the road is necessary for accessing resources and reproduction. Thus, failure to direct movement to safe crossing locations can be counterproductive to conservation efforts.

Our results also indicate the probability of trespassing a fence-end treatment increases the longer an amphibian spends attempting to navigate to an opening in the fence structure.

TABLE 3 Model selection for the fence arena experiment for all amphibians along state route 78 in Athens County, Ohio near the city of Nelsonville.

	Model	Predictors	AICc	ΔAICc	W
1	Exp	TX + TT	247.3	0	0.44
2	Single	TX	249.0	1.66	0.19
3	Env + Exp	°C + TX	249.3	2.01	0.16
4	Single	TT	251.7	4.34	0.05
5	Single	°C	251.8	4.51	0.05
6	Null	~	252.8	5.51	0.03
7	Env + Exp	°C + PR + TS + TX	253.1	5.77	0.03
8	Single	TS	254.2	6.84	0.03
9	Single	PR	254.8	7.43	0.01
10	Singe	EX	254.8	7.49	0.01
11	Env	°C + PR + TS	255.3	7.92	<0.01
12	Single	SX	256.2	8.91	<0.01
13	Single	G	257.3	9.94	<0.01
14	Exp + Ind	TX + TT + SX + G + EX	257.5	10.20	<0.01
15	Global	°C + PR + TS + TX + SX + G + EX	258.3	10.96	<0.01
16	Ind	SX + G + EX	261.1	13.79	<0.01
17	Env + Ind	°C + PR + TS + SX + G + EX	262.7	15.35	<0.01

Model statistics include AICc, Akaike information criteria, the difference between the best supported model and other models (ΔAIC), and model weights (W). The response variable was measured as success (moved around the non-treatment side of the fence arena; coded as 0) or fail (escaped the fence arena by circumventing the experimental fence-end treatment; coded as 1). The predictor variables include, TX, treatment; TT, total length of trail; °C, temperature; PR, precipitation rate; TS, time of trial since sunset; EX, experience; SX, sex; G, genus.

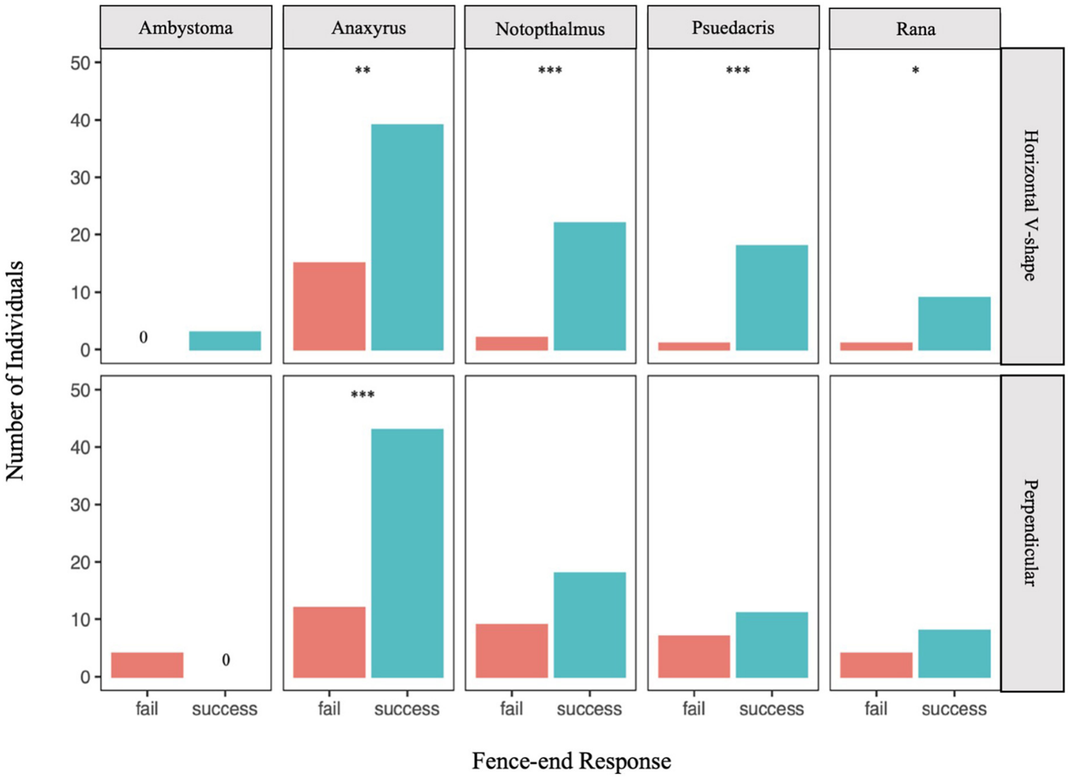

FIGURE 3
Number of individual amphibians that successfully responded to the fence-end treatment by moving around the side of the fence that remained unobstructed (success) or failed to respond by moving around the experimental fence-end treatment (fail). Asterisks denote significance level of the *G*-test with a Yates correction: $^{*}p < 0.05$, $^{**}p = 0.001$, $^{***}p < 0.001$.

Therefore, transportation agencies should be cautious of designing and implementing extended fences as a measure to combat the fence-end effect beyond the distance amphibians will approach a fence-end treatment and return to the eco-passage. Although it is beyond the scope of this study, further investigation of various fence lengths to determine the maximum effective distance between an eco-passage and a fence-end treatment, where an amphibian is still willing to return to the eco-passage, would be valuable and could influence important management recommendations for future road projects. In our study, when placed within 1 m of a fence-end treatment we found 16% of amphibians circumvented the experimental fence-end treatment and 33% either abandoned the arena or became trapped at the fence. Therefore, when the distance an amphibian must traverse along a fence wall before it encounters a fence-end treatment increases, failure to respond to a fence-end treatment should also be expected to increase. Thus, amphibians may show a higher propensity to circumvent the fence-end treatment rather than retrace their movements back toward the eco-passage. Further consideration is also required when considering extending fence length as a conservation measure to combat the fence-end effect because it may subsequently escalate the barrier effect of fences, which occurs when amphibians forgo breeding opportunities by abandoning migration efforts and returning to terrestrial habitats when they are unable to locate an opening to cross through (Jaeger and Fahrig, 2004; Matos et al., 2019). One solution for longer terminal fences is to angle the terminal fence segment toward the terrestrial or wetland habitat with the fence ends diagonal to the eco-passage to create a gradient that funnels animals in the direction of the eco-passage, opposite from the fence ends (Allaback and Laabs, 2002; Pagnucco et al., 2012). Thus, fence extensions to dilute the fence-end effect could be permissible under this scenario. However, this possibility remains untested, and in many cases, fences are constructed parallel to the road as landscape features such as ditches, hillsides, and private property often restrict fence design options. Ottburg and van der Grift (2019) suggested "tailor-made measures" that include increasing the number of eco-passages based on mean movement distance of different species and then installing fences beyond the location of the migration corridor. This is certainly a viable option, particularly if eco-passages are constructed at the edge of a well-defined migration corridor. We expect that mitigation structures constructed under this scenario to potentially eliminate most of the issues associated with the fence-end effect. Until further research is conducted to determine optimal fence length between a tunnel and fence-end treatment for amphibians, we suggest following suggestions of Broekmeyer and Steingröver (2001). The authors recommend tunnels should be built at distances up to 0.25 × the dispersal distance of the target species. However, for terminal fences, we would need to adjust for the target species return to the eco-passage, thus we suggest that fence length not exceed 0.125 × dispersal distance or mean movement distance (if known) of the target species. For existing mitigating structures that require retrofitting or new mitigation structures that require longer fences to span the entire length of a migration corridor, we also encourage installing multiple short horizontal v-shape barriers bent inward toward the eco-passage throughout the entire span of the terminal fence. By segmenting a terminal fence in this way, the v-shaped barriers can reduce the time and distance an amphibian would take to walk in the wrong direction until it eventually encounters a fence-end treatment. This would balance the need for longer fences by creating shorter segments within it, thus making the fences more cost-efficient. Furthermore, this fence design also accounts for multiple species with different movement capacities (Hels and Buchwald, 2001), thus expanding conservation efforts to protect the local community of amphibians rather than a single target species.

Furthermore, our data indicates that the probability of success may increase with warmer temperatures. Despite our attempt to isolate behavioral responses to alternative fence-end treatments under natural migratory conditions, COVID-19 restrictions placed on logistics, personnel, and work schedule, prohibited testing with prolonged breeders such as *N. viridescens*, *P. crucifer*, and *Rana* spp. when they are most active (Pfingsten et al., 2013). Although we were unable to capture the full migratory season for some genera, our data indicates the probability of success improves with warmer temperatures. Thus, we expect late season breeders (April to May) to display more willingness to respond to the fence-end treatment compared to their early breeding counterparts based on the positive trend observed between February and March. For this reason, future road mortality studies should consider seasonal effects when reporting roadkill frequencies when the fence-end effect is detected. Additionally, we were unable to test fence-end treatments for non-breeding adults or dispersing juveniles. Prior fence experience ranked low in our model selection, suggesting amphibian response to a fence-end treatment is not influenced by previous experiences. Thus, adults returning to overwintering sites may respond differently to alternative treatments. Furthermore, variation in treatment response between breeding and non-breeding individuals (e.g., subadults, juveniles) has not been well studied in road ecology literature but could influence the design and implementation of mitigation fences (e.g., whether the fence is built on the aquatic or terrestrial side of the road).

In testing for alternative behavioral responses to two different fence-end treatments, we found only *A. americanus* showed a similar response to both the horizontal v-shape and perpendicular treatment. In contrast, *N. viridescens*, *P. crucifer*, and *Rana* spp. only showed a successful response to the horizontal v-shape treatment, but not the perpendicular treatment. Additionally, due to the small sample size, no significant difference was found for *Ambystoma* spp. However, all test individuals failed to respond to the perpendicular fence-end treatment; in contrast all test individuals successfully responded to the horizontal v-shape fence-end treatment, suggesting the horizontal v-shape fence-end treatment may be a better option for these species as well. Overall, our study indicates the horizontal v-shape fence-end treatment is a more effective measure to mitigate for the fence-end effect compared to the perpendicular fence-end treatment for a variety of migratory amphibian species. Our results corroborate a study by Dodd et al. (2004) on mitigation structures consisting of perpendicular fence-end treatments. The authors found road mortality increased at the fence ends by 109 and 300%, respectively, suggesting perpendicular fence-end treatments are not optimal for amphibians, reptiles, or mammals. In contrast, Markle and Stapleton (2022) found that road mortality frequencies did not increase at the end-fence after installing j-shaped fence-end treatments (e.g., a horizontal v-shape fence-end treatment with a rounded corner), suggesting the treatment effectively prevented turtles from escaping the mitigation system and returned animals back to the habitat they emerged from. It is unclear what structure attributes contribute to the higher rate of success observed for the horizontal v-shape fence-end treatment for amphibians; however, we observed amphibians were more likely to turn around after becoming trapped in the corner created by the sharper angle, which may significantly decrease the fence-end effect but could also act as a prey-trap (Baxter-Gilbert et al., 2015). With few exceptions (Ford and Clevenger, 2010), little attention has been paid to examining predation events along fences; however, several studies evaluated the prey-trap hypothesis in eco-passage have largely found this risk to be minimal (Little et al., 2002; Ford and Clevenger, 2010). Furthermore, it is unclear if the angle of the fence-end treatment is the important factor influencing amphibian response compared to other potential structure attributes, such as the length of the fence-end treatment or if a rounded corner like the j-shape would perform better than the horizontal v-shape. In addition, our study suggests for some amphibian species (*N. viridescens*, *P. crucifer*, and *Rana* spp.) the angle of the fence-end treatment may be a sufficient measure of success given effectiveness improved when the fence ends were bent further inward at a sharper 45° angle. However, it remains unclear if a 45° angle is the optimal design for these species or if, for example, this angle represents a minimum or maximum limit above or below which the treatment becomes less effective. Thus, given the conservation crisis of protecting amphibian populations against local extirpation resulting from additive road mortality, asserting optimal design features is critical and requires further research attention.

Despite our attempts to isolate migratory amphibian response to alternative fence-end treatments, additional insights, and evidence are needed to optimize structure design for amphibians. Nevertheless, our study illustrates an experimental approach to begin resolving fence attributes for migratory amphibians, providing a general guideline for fence-end treatments. More specifically, the fence-end effect can be mitigated for by installing fence-end treatments and that these structures should be bent inward to guide amphibians away from the road and to eco-passages, preferably in the horizontal v-shape for migratory amphibians that require movement through associated crossing structures.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

This animal study was reviewed and approved by the Ohio University Animal Care and Use Committee.

Author contributions

KH planned and provided the logistical support that shaped the research. KH and KO conducted the fieldwork and data collection. KH and VP carried out the statistics analysis and were involved in writing the manuscript and providing critical feedback. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Ohio Department of Transportation (ODOT), the Federal Highway Administration (Agreement #30218 SJN #135504), funded the fieldwork and equipment and Ohio University, Graduate College funded the materials used to make the experimental arenas.

Acknowledgments

We thank the Ohio Department of Transportation (ODOT), the Federal Highway Administration, and Ohio University for funding our research. We are grateful for the support of Drs. Molly Morris, Shawn Kuchta, and Willem Rosenberg for helpful advice and support. We thank to Dr. Shawn Kuchta for also assisting with the construction and maintenance of the experimental arenas. We are particularly grateful to our field volunteers, Jane Armstrong, Brennan Brindley, David Cole, and Alexa Tanner for extensive data collection. Special thanks to Julia Joos, Meredith Fitschen-Brown, and Mattea Lewis for assisting with data collection during a particularly explosive migration night. We also sincerely appreciate all the time and effort of the reviewers who provided valuable comments and suggestions on our manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1008462/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fevo.2023.1145322

OPEN ACCESS

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RECEIVED 16 January 2023
ACCEPTED 17 April 2023
PUBLISHED 24 May 2023

CITATION
Brehme CS, Barnes S, Ewing B, Gould P, Vaughan C, Hobbs M, Tornaci C, Holm S, Sheldon H, Fiutak J and Fisher RN (2023) Elevated road segment (ERS) passage design may provide enhanced connectivity for amphibians, reptiles, and small mammals. *Front. Ecol. Evol.* 11:1145322.
doi: 10.3389/fevo.2023.1145322

Elevated road segment (ERS) passage design may provide enhanced connectivity for amphibians, reptiles, and small mammals

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Introduction: Designs for safe and effective road crossing structures for small animals are typically under-road microtunnels and culverts which have varying levels of effectiveness reported in the scientific literature. Many species, particularly migratory amphibians, may have limited ability to find and use passages if they are too far apart, resulting in substantial barrier effects.

Methods: We designed a novel open elevated passage (elevated road segment: ERS), similar to a low terrestrial bridge, that could theoretically be built to any length based upon species needs and movement characteristics. A 30 m length prototype ERS was installed along a forest road with a history of amphibian road mortality in Sierra National Forest, Fresno County, CA, USA. From 2018 to 2021, we monitored small animal activity under the ERS in relation to surrounding roadside and forest habitats using active infrared cameras.

Results: We documented a total of 8,815 unique use events, using species specific independence criteria, across 22 species of amphibians (3), reptiles (4), and small mammals (15). Poisson regression modeling of taxonomic group activity under the ERS, roadside and forest, showed that amphibian activity was highest in the forest habitat, no differences were observed for reptiles, and small mammal activity was highest under the ERS. However, mean activity estimates under the ERS were equal to or greater than the open roadside habitat for all 22 species, suggesting that adding cover objects, such as downed logs and vegetation may further enhance passage use.

Discussion: Overall, results showed that the design of the ERS crossing has potential to provide high connectivity for a wide range of amphibian, reptile, and small mammal species while reducing road mortality. ERS systems can also be used in areas with challenging terrain and other hydrological and environmental constraints. Incorporating current road ecology science, we provide supplemental ERS concept designs for secondary roads, primary roads and highways to help increase the options available for road mitigation planning for small animals.

KEYWORDS

road ecology, road crossing, passage, herpetofauna, design, tunnel, toad, salamanders

1. Introduction

Directly and indirectly, roads represent a substantial threat to wildlife at individual, population, and meta-population levels (e.g., Forman et al., 2003; Andrews et al., 2015; van der Ree et al., 2015). Directly, roads affect wildlife via road-associated mortality, which can threaten population persistence by reducing survivorship (Gibbs and Shriver, 2002; Ceia-Hasse et al., 2018; Barrientos et al., 2021). Indirectly, species behavioral avoidance of roads and altered roadside habitats can reduce population connectivity resulting in habitat fragmentation effects (Jackson and Fahrig, 2011; van der Ree et al., 2015; D'Amico et al., 2016). Not all roads are equal in their impacts, but negative impacts have been documented from low-use unpaved roads to high-use multi-lane highways for many wildlife species (Goosem, 2002; Forman et al., 2003; Andrews et al., 2008; Dean et al., 2019).

Species at greatest risk of negative road impacts are those with large movement distances or home ranges that encounter roads as part of daily, seasonal, or annual movements, those that are slow moving, and those that do not exhibit road or vehicle avoidance behaviors (Jaeger and Fahrig, 2004; Glista et al., 2008; Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2012, Brehme et al., 2018). For example, many reptiles and amphibian species are particularly susceptible to the negative impacts from roads as they slowly migrate among different habitats to meet their basic life history requirements, such as breeding, development, foraging, and overwintering (e.g., Gibbs and Shriver, 2002; Andrews et al., 2008; Beebee, 2013). Many reptiles may be attracted to the road surface to thermoregulate (Jochimsen et al., 2014). Similarly, amphibians often do not avoid roads or may be attracted to roads during or after rainfall to easily absorb surface water through their permeable ventral skin (Beebee, 2013). Once on roads, they often "freeze" in response to oncoming traffic, further exacerbating the risk of road mortality (Andrews and Gibbons, 2005; Mazerolle et al., 2005). When roads intersect vital habitats for these species, it can result in reduced gene flow, lower population sizes and increased probability of extirpation (e.g., Gibbs and Shriver, 2002; Semlitsch, 2008; Van Buskirk, 2012; Beebee, 2013; Brehme et al., 2018).

Road avoidance behaviors that can fragment populations have also been documented for reptiles and small mammals. For instance, Andrews and Gibbons (2005) found that smaller snakes were much more likely to avoid roads than larger snakes. Similarly, smaller mammals, particularly closed-habitat specialists, are more likely to avoid roads of varying traffic volumes, and there is evidence that multiple species may be repelled by paved road substrates (Goosem, 2002; McGregor et al., 2008; Brehme et al., 2013; Ascensão et al., 2016; Chen and Koprowski, 2019).

Finally, many herpetofauna species migrate among wide swaths of terrestrial and aquatic habitats using straight line trajectories (Matthews and Pope, 1999; Sinsch, 2006; Liang, 2010; Southwood and Avens, 2010), and small mammals move among suitable patches of habitat or along road verges (Gunson et al., 2011). Therefore, road mortality for many small animals is often not concentrated at a particular corridor or "hot spot." Instead, high roadkill numbers are often spread along many meters or kilometers of roads (Glista et al., 2008; Matos et al., 2012; Garrah et al., 2015).

Current practice to mitigate negative impacts on migrating amphibians and other small animals is to install one or several small tunnels under the roadway with stand-alone barrier fencing to help lead animals to the passages (Schmidt et al., 2008; Andrews et al., 2015; Langton and Clevenger, 2021). However, there is still a need for more studies on their effectiveness (Lesbarreres and Fahrig, 2012; Beebee, 2013; Langton and Clevenger, 2017). Recent studies have also found that migrating amphibians will turn around after a short distance if they do not readily find a passage after encountering road barrier fencing. For instance, migrating common toads (*Bufo bufo*), California tiger salamanders (*Ambystoma californiense*), and Yosemite toads (*Anaxyrus canorus*) have average "give-up" distances of 40–50 m along road fencing although they migrate a kilometer or more (Ottburg and van der Grift, 2019; Brehme et al., 2021, 2022a). This behavior can result in a "filter effect" that could be more detrimental to the population than road mortality by substantially reducing connectivity and breeding opportunities (Allaback and Laabs, 2002-2003; Ceia-Hasse et al., 2018; Ottburg and van der Grift, 2019). "Give-up" distances directly inform passage spacing as distance between built passages can be optimized for differing target levels of permeability. To our knowledge, it is unknown if this phenomenon occurs with reptiles and small mammals, although for non-migratory species, passage spacing greater than the width of a core home range would be expected to filter movements among individuals of populations separated by road fencing.

Installing passages underneath the road surface with raised barrier fencing often requires some grading and recontouring slopes to the passage entrance(s) which can potentially damage natural resources or change hydrological flows. Considering these factors and limitations at our study site, we designed a novel small animal road passage we call an elevated road segment (ERS). The ERS is an elevated road passage that can be built to any length and is permeable to light and rainfall, allowing for a wetted passage for amphibians moving during rainfall events. The ERS prototype was installed directly on top of a forest road with a history of substantial amphibian road mortality, particularly of federally threatened Yosemite toads (Vaughan et al., 2023), in Sierra National Forest, Fresno County, CA, USA. Although Yosemite toads were the impetus of this project, SNF harbors a wide variety of amphibians, reptiles, and small mammals that may be at risk from road related impacts.

The first objective of this study was to evaluate the efficacy of the ERS by monitoring the relative activity of amphibians, reptiles, and small mammals under the ERS, in comparison to adjacent roadside habitat and interior forest habitat. We postulated that any activity under the ERS would represent some level of permeability to movement for small animal species and that equal levels of activity between the ERS and adjacent habitat would represent high levels of permeability to movement, suggesting no avoidance. If the ERS was effective, our second objective was to provide suitability considerations and engineered concept designs for transportation planners and conservation agencies to apply the open ERS passage concept to a wide variety of road types (low-use unpaved roads to high-use highways).

2. Materials and methods

2.1. Study site

Our study took place on US Forest Service (USFS) road 9S09 in Sierra National Forest, Fresno County, CA, USA from 2018 to 2021 (**Figure 1**). The road was the focus of mitigation as it bisects breeding meadow and upland habitat for the federally threatened Yosemite toad (Brehme et al., 2022a; Vaughan et al., 2023). The conifer forest is primarily dominated by lodgepole pine and wet meadow habitat that include sandy lupine areas, herbaceous plants, rocky outcroppings, rotting tree stumps, fallen logs, and leaf litter.

In June 2018, the ERS was installed on top of a solid impervious aggregate roadbed (USFS Rd. 9S09). The prototype ERS is a 30 m long and 4.9 m wide road crossing structure that was placed on top of the existing road surface and is composed of hardwood laminated billet road mats that are designed for use by heavy equipment at construction sites (Emtek®; **Figure 2**). The road mats are approximately 15 cm thick, installed on top of 20 cm (8 in.) high laminated billet support bars placed approximately 2 m apart directly on and perpendicular to the road, that allows for passage of small animals underneath. In addition, the ERS is permeable to light and rainfall, allowing a wetted passage for amphibians moving during rainfall events. It was built to meet codes and specifications for USFS, County, and City roads and can theoretically be built to any length, width, or at increased heights depending upon the size of the supports used.

Approximately 120 m lengths of polymer mesh and solid barrier fencing were installed along both sides of the road leading to the ERS passage [ERTEC® rigid polymer matrix E-Fence™ and Animex® high-density polyethylene (HDPE-2)]. Jump-outs (ERTEC® cones and high berms) were installed a minimum of every 10 m along the fence to provide toads and other small vertebrates opportunities to safely return into the habitat if they incidentally accessed the road within the barrier fencing. The first 15 to 20 m of fencing connected to the ERS passage spanned roadside habitat that was clear of most trees and leaf litter, while the remaining fencing spanned out into forested habitat. At outer fence ends, turnarounds were installed to redirect animals away from the road and back toward the habitat in a U-shaped fashion. The turnarounds were approximately 2 m long and 1 m in width. Fencing was installed with the bottom buried in the ground according to manufacturer's guidelines. The length of the fencing is longer than would be recommended for high permeability of toad movement on each side of the passage; however, this was done as part of a concurrent study on fence movement behavior for the Yosemite toad (Brehme et al., 2022a).

2.2. Study design

A total of 18 HALT® active-trigger camera systems (Hobbs and Brehme, 2017) were used to monitor small animal activity along the fencing in the habitat leading to the ERS and then immediately adjacent and under the ERS. The system uses an active infrared trigger beam integrated into a standard 37 cm threshold so that when animals pass over the threshold and intercept the beam, the camera is triggered. All cameras were programmed to take a short video or 3 rapid-fire photos with a 5 s interval set between possible trigger events.

Fourteen cameras were placed along fencing on one side of the road (west) every 20 m and at the end of fence end turnarounds (**Figure 3**). The cameras were set only along the west fence line that contained forest habitat adjacent to a Yosemite toad breeding meadow. Fence camera placements and differences in barrier fence types also supported a concurrent study on Yosemite toad fence movement distances as they migrate from the meadow (Brehme et al., 2022a). The number of cameras available did not support monitoring both sides of the road. In 2019, we added an additional camera between the 2 cameras closest to the ERS on each side to increase sampling of adjacent roadside habitat. The closest cameras to the ERS along the fence were approximately 8 m from each side of the ERS opening to shield the cameras from the view of forest visitors.

The expansive length of the ERS underpass made it impossible to sample completely. We placed one HALT camera system under both sides of the ERS, where it intersected with the fence line, to best capture photos of animals that entered the ERS after moving along the fence. After the first year, we used triggers with an extended 1 m trigger length to better cover the passage opening and to help capture images of animals that did not take a hard turn into the structure. Therefore, we targeted animals moving along the fence line and directly into the structure. The under ERS camera triggers were placed a minimum of 30 to 60 cm deep into the passage to ensure animals were fully under the ERS. Additional HALT cameras and triggers were added within the same passage segments in 2020 and 2021 for better coverage (Brehme et al., 2022a), but these were not used in the analysis due to very low activity, as most animals crossed close to the fence line. Finally, we also installed eight Reconyx cameras (factory set with 3–5 m focal distances with a 60-degree field of view) under multiple subsections of the east side of the ERS (facing west); they were set to a time lapse of every 5 min to gather more data on Yosemite toad specific movements. Due to the vast number of photos generated by these cameras, they were only analyzed only for Yosemite toads during rain events and during times when toads were detected on habitat cameras along the fence line.

Each year, all cameras were set as soon as the snow melted and the road opened. Cameras were checked and reset on a weekly

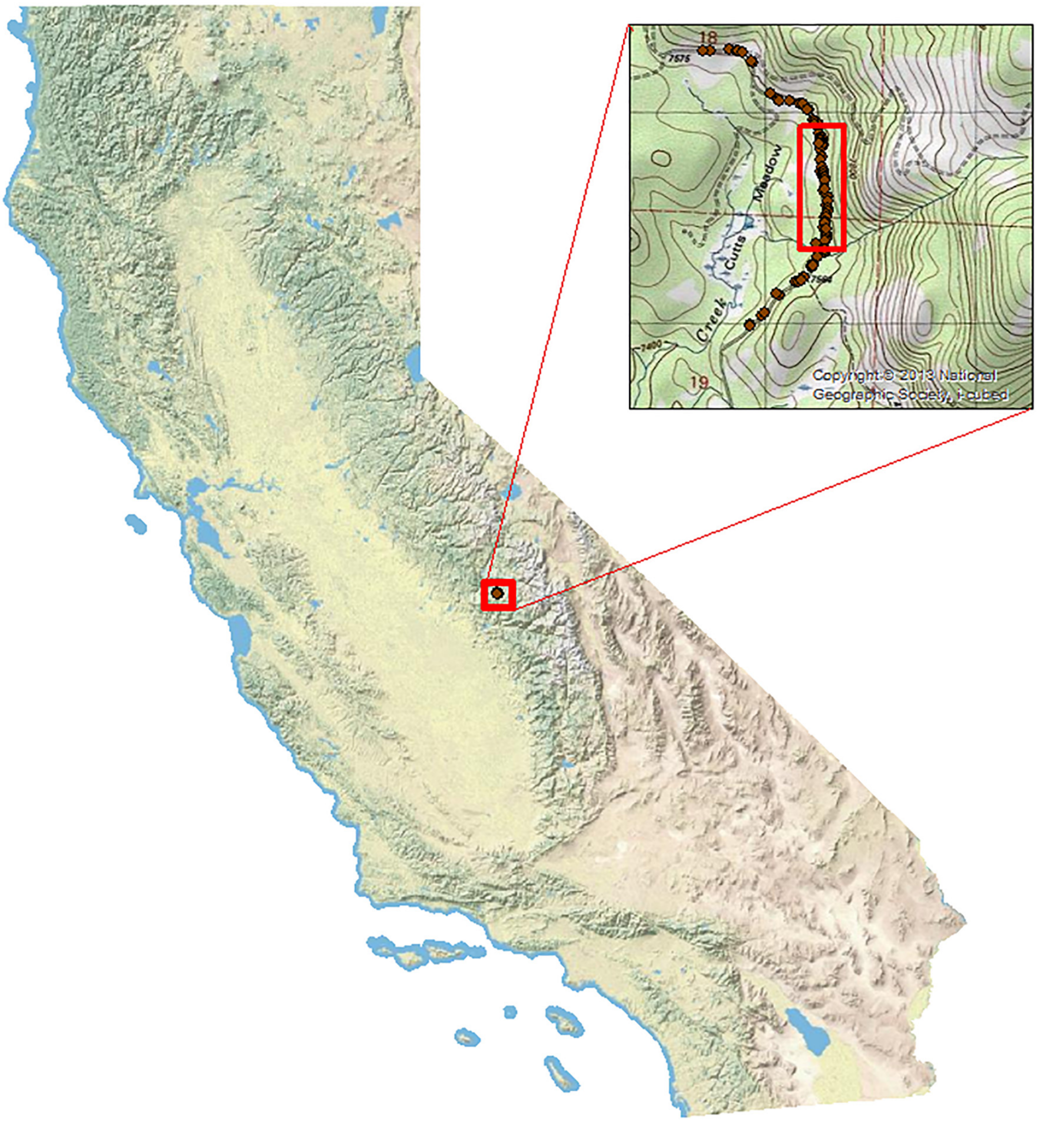

FIGURE 1
Study area in Sierra National Forest, Fresno County, CA, USA. Dark red points represent Yosemite toad mortalities in 2017 prior to installation of the elevated road segment (ERS) and barrier fencing (footprint designated by red open rectangle).

basis during the late spring and summer (May–October 2018, July–October 2019, June–September 2020, May–October 2021), and ran for a total of 10,546 days. Total summer precipitation in nearby Huntington Lake during the monitoring periods was approximately 23 mm for 2018 (June–October), 33 mm for 2019 (July–October), 10 mm for 2020 (June–September), and 18 mm for 2021 (May–October) after the snow melt (California Nevada River Forecast Center).[1] Summer seasons were approximately 76 mm or more below average rainfall during these periods (Western Regional Climate Center 044176-5).

In 2020, cameras were removed in early September due the “Creek Fire,” which burned almost 400,000 acres in Sierra National Forest and right up to the edge of the Yosemite toad breeding meadow adjacent to our study site. After the fire, as part of suppression repair work, the USFS piled downed wood and debris next to the study area and close to the fence line. In addition to below normal rainfall, it is unknown how these factors affected small animal activity or numbers. Road mortality surveys were also conducted along a 2 miles section of 9S09 that encompassed the ERS study site by the USFS in all years (Vaughan et al., 2023).

2.3. Analysis

All photos from the HALT cameras were reviewed and animals identified to species. Due to the extremely large number of time-lapse photos from Reconyx cameras, all photos that were within 1 h of any Yosemite toad HALT camera detection were reviewed. Therefore, time lapse Reconyx camera data was specific for Yosemite toad use only.

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[1] https://www.cnrfc.noaa.gov/monthly_precip_2021.php

FIGURE 2
Diagram **(A)** and photos **(B)** of elevated road segment in Sierra National Forest, Fresno County, CA, USA.

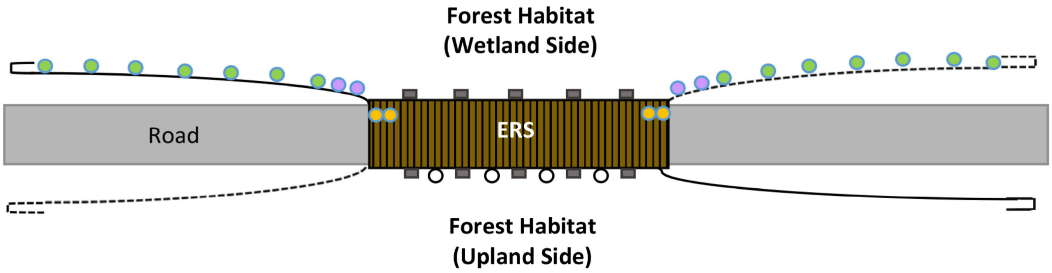

FIGURE 3
Schematic of elevated road segment (ERS), HALT cameras (circles: green = forest, purple = roadside habitat, and yellow = under ERS), and time-lapse cameras (black open circles; 4 shown of 8); not to scale.

To assess relative ERS permeability for all species, results were analyzed for 3 general locations: (1) under the ERS passage, (2) along the roadside habitat immediately outside the ERS passage (within 0–28 m of passage and <5 m from the road), and (3) within the interior forest habitat approximately 28 m from passage and >5 m from road). Roadside habitat designation was based on a general lack of ground cover on the west side of the road immediately adjacent to the ERS, in comparison to abundant trees, shrubs, woody debris, and leaf litter at approximately 5 m and beyond. We identified all animals to species or genus and processed images into animal records using CamtrapR v. 2.2.0.7 (Niedballa et al., 2016). We conservatively considered images for non-migrating species to represent unique, independent use events when a minimum of 30 min had passed between the last unique observation of a species at the same camera (Si et al., 2014; Laughlin et al., 2020; Vilella et al., 2020). For Yosemite toads migrating from wetland to upland habitats, we used a 5-min window to reduce the likelihood of excluding multiple individuals moving in pulses such as during rainfall events. For modeling, species counts represented the sum of unique use events per year at each camera location. Controlling for variation in temporal activity among years, abundance of use (hereby referred to as "activity") was a relative measure of frequency of space-use by each species (Gilbert et al., 2021) and represented general patterns in species activity across spatial and temporal gradients.

To analyze the use of the ERS by amphibians, reptiles, and small mammals, we modeled the activity at each individual camera using Poisson regression models with Bayesian inference (Kery et al., 2009; Yamaura et al., 2016; Gould et al., 2022). Our observed data are y_{ikt}, where y is the count (Poisson regression) of species *k*, use events at camera *i*, in year *t*. In contrast to Brehme et al. (2022a), where an n-mixture model was used to analyze activity data, Poisson regression does not include a model for observation process, as these data may violate population closure assumptions required for N-mixture models (Royle, 2004). Poisson regression remains a robust tool for estimating relative abundance when N-mixture model assumptions are not met (Barker et al., 2018). Active trigger cameras are not subject to differences in detection probability due to variations that are inherent in passive infrared cameras (Hobbs and Brehme, 2017), and differences in camera activity that could lead to increased counts were accounted for by effort (number of days active). Therefore, beyond these, we assumed all cameras have equal detection probabilities, and therefore, patterns in activity observed here are not systematically biased.

For the community Poisson regression, the relative abundance model for counts y_{ikt} is defined:

$$y_{ikt} \sim Poisson\left(\lambda_{ikt} \times w_{it}\right)$$

where w indicates if camera i was active during year t, and λ_{ikt} is the rate parameter of species k at camera i, during year t. We estimated λ_{ikt} with a log link-function and included the covariates about camera location and days of camera activity (effort):

$$log\left(\lambda_{ikt}\right) = \beta_{0k} + \beta_{roadside,k} \times Roadside_i + \beta_{forest,k} \times Forest_i$$

$$+\beta_{effort,k} \times effort_{it} + \varepsilon_{ikt}$$

where each covariate now corresponds to the change in activity on the log scale. In this case, β_{0k} corresponds to the relative activity of animals for cameras under the ERS, while $\beta_{roadside,k}$ and $\beta_{forest,k}$ represent the relative effects of roadside and forest habitats on activity, respectively. The parameter $\beta_{effort,k}$ accounts for differences in effort among camera location and years. We also included an observation-level random-effect, ε_{ikt}, which is useful in accounting for over-dispersion present in the data (Harrison, 2014).

All model parameters were specified as either centered or non-centered random effects, with shared precision parameters. Specifically, we specified the prior for β_0 as being a non-centered, normally distributed random prior.

$$\beta_{0k} \sim normal\left(0, \sigma_0\right)$$

$$\sigma_0 \sim uniform\left(0, 10\right)$$

All other beta parameters were specified with centered, normally distributed priors, centered on parameter-specific hyper-parameter means and standard deviations.

$$\beta_{nk} \sim normal\left(\mu_n, \sigma_n\right)$$

$$\mu_n \sim normal\left(0, 3.16\right)$$

$$\sigma_n \sim uniform\left(0, 10\right)$$

Finally, we specified the prior for the observation-level random-effect, ε_{ikt}, to be normally distributed with a mean of zero and a gamma distributed standard deviation, with shape and rate parameters of 0.9.

TABLE 1 Counts of unique images for each species in each year of sampling.

		Year				
		2018	2019	2020	2021	Sum
Amphibians	Pacific tree frog *Pseudacris regilla*	755	164	73	168	1,160
	Sierra Nevada ensatina *Ensatina eschscholtzii platensis*	116	27	2	9	154
	Yosemite toad *Anaxyrus canorus*	109	78	9	5	201
Mammals	American marten *Martes americana*	0	11	0	2	13
	Broad-footed mole *Scapanus latimanus*	0	0	1	0	1
	Bushy-tailed woodrat *Neotoma cinerea*	0	0	9	24	33
	California ground squirrel *Otospermophilus beecheyi*	1	0	1	6	8
	Douglas squirrel *Tamiasciurus douglasii*	152	99	84	87	422
	Golden-mantled ground squirrel *Callospermophilus lateralis*	3	0	4	15	22
	Lodgepole chipmunk *Neotamias speciosus*	78	8	128	139	353
	Long-tailed weasel *Mustela frenata*	5	0	9	3	17
	Mountain pocket gopher *Thomomys monticola*	13	5	25	27	70
	Northern flying squirrel *Glaucomys sabrinus*	0	4	1	6	11

(Continued)

TABLE 1 (Continued)

		Year				
		2018	2019	2020	2021	Sum
	Peromyscus mice species *Peromyscus* spp.	1,804	585	1,378	1,330	5,097
	Shrew *Sorex* spp.	123	38	122	67	350
	Spotted skunk *Spilogale gracilis*	2	2	0	0	4
	Vole spp. *Microtus* spp.	134	19	98	58	309
	Yellow-bellied marmot *Marmota flaviventris*	19	3	10	0	32
Reptiles	Mountain garter snake *Thamnophis elegans elegans*	97	76	19	25	217
	Rubber Boa *Charina bottae*	53	35	40	15	143
	Sierra alligator lizard *Elgaria coerulea*	58	27	44	28	157
	Western fence lizard *Sceloporus occidentalis*	11	13	13	39	76

The sum column corresponds to the total number of images recorded for each species that were separated by 30 minutes between image captures for non-migratory species and 5 min for the migratory Yosemite toad.

Models were run in JAGS v 4.3.0 (Plummer, 2003) using R v. 4.1.2 (R Development Core Team, 2013) with package jagsUI (V 1.5.2; Kellner, 2015). Models were run for 100,000 iterations with an adaptation of 1,000 iterations, a burn-in of 1,000 iterations, and a thinning rate of 5, across 6 chains, yielding a total of 59,400 posterior samples. We visually inspected trace plots for model convergence and considered all parameters to have converged when Gelman–Ruben values were <1.10. We examined model explanatory power by comparing Freeman-Tukey residuals for the observed and model generated dataset. We calculated a Bayesian *P*-value for the model by calculating Freeman-Tukey residuals across sites, years, and species. Values close to 0.5 indicate high explanatory power. We summarized all parameters using 90% Highest Density Intervals (HDI), and effects were considered strong if HDI intervals did not include zero. We also report Poisson rate ratios (relative species activity in forest or roadside in comparison to under ERS) and effects were considered strong if HDI intervals did not include one.

3. Results

Across 4 years of sampling comprising 10,546 camera trap 24-h periods (cameras x trap periods), we observed 8,850 unique use events, given our species specific independence criteria. These included 6,742 observations of mammals, 1,515 observations of amphibians, and 593 observations of reptiles, across 22 species (Table 1). The most common species included *Peromyscus* mice (*Peromyscus* spp., n = 5,097) and Pacific tree frogs (*Pseudacris regilla*, n = 1,160). Lower numbers of animals in general were documented in 2019, likely due to the shorter monitoring period that year, from a late snow melt and other factors.

3.1. Activity patterns

Activity patterns of the species-groups within our study generally fit either nocturnal or diurnal activity (Figure 4). All three species of amphibians, 7/15 species of mammals, and 1/4 reptiles exhibited nocturnal activity patterns. The remaining reptiles and mammal species exhibited more diurnal activity patterns, although Douglas squirrel activity exhibited a peak near sunrise, consistent with crepuscular activity. Spatially explicit species count indices in relation to unique camera locations for each species are shown in Figure 5.

3.2. Poisson count model

Overall, the Poisson model showed animal activity was strongly associated with location and effort (Table 2). The community-wide response to roadside habitat was significantly negative, indicating roadside habitat had less overall species activity compared to under the ERS (Table 3). There was also evidence that overall activity was lower in the interior forest, compared to under the ERS (0.69×, 0.39–0.98); however, there was considerably more uncertainty (SD = 1.10) compared to roadside habitat (SD = 0.35) due to species specific responses. Additionally, the community-wide response to camera effort was significantly positive, as increased active time allowed for more observations, and the responses were relatively consistent across species (SD = 0.54).

Within the community, significant taxonomic patterns were present in response to location (Figure 5 and Table 3). Mammal activity was significantly lower in both roadside (mean = 0.53×, 90% HDI: 0.39-0.68) and interior forest (0.52×, 0.37–0.66), in comparison to under the ERS. Amphibians (0.71×, 0.44–0.98) and reptiles (0.60×, 0.42–0.78) were less active in roadside habitat than ERS. Additionally, amphibians were significantly more active in the forest than under the ERS (1.68×, 1.00–2.22).

All mammal species exhibited higher mean activity estimates within the ERS than roadside habitat (Figure 6 and Table 3). Further, 4/14 species (long-tailed weasel, mountain pocket gopher, northern flying squirrel, and voles) had higher mean activity in the forest, although not significantly more, compared to under the ERS. Relative to the taxonomic average effects of location, 5 species (bushy-tailed woodrat, California ground squirrel, chipmunk, Douglas squirrel, and golden-mantled ground squirrel) exhibited less activity in forest than under the ERS (Figure 6 and Table 3).

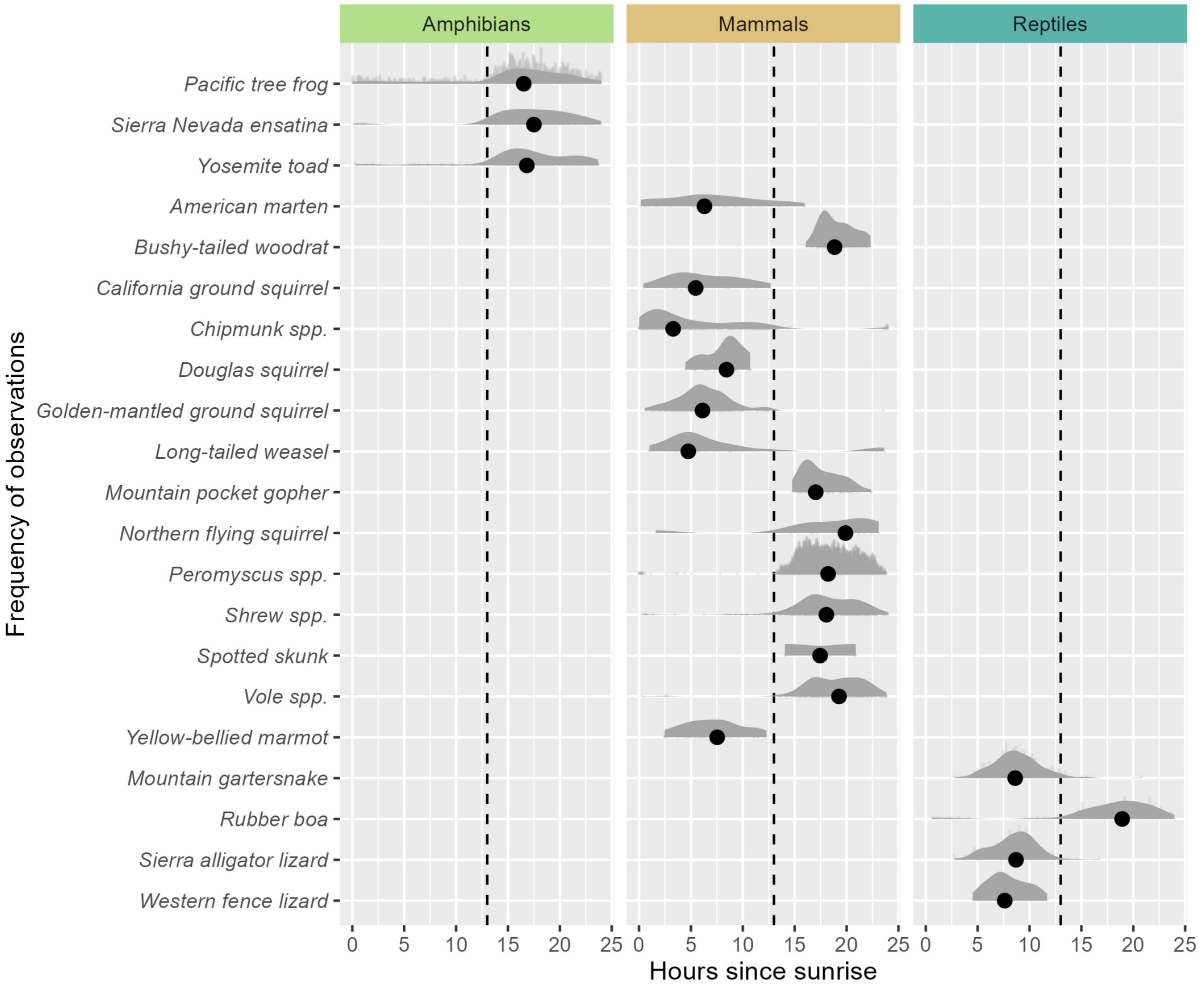

FIGURE 4
Temporal activity patterns of animals detected represented by scaled density. Sunrise times are based on 6:00 A.M., PST and represent an approximation of when diurnal or crepuscular activity may be expected to be observed. The dashed line indicates 7:00 P.M. PST and represents an approximate average sunset time across the study period. Activity density curves to the left of the dashed line indicate animals with diurnal activity. Activity density curves to the right of the dashed line indicate animals with more nocturnal activity patterns. Dots represent the mean time-since-sunrise of all observed images.

Amphibian activity patterns were more consistent than patterns observed for mammals. All three species were, on average, more active in the forest than in roadside habitat (Figure 6 and Table 3). Although no amphibian species exhibited significantly greater activity within the forest than under the ERS, the taxonomic average activity for amphibians was significantly greater in the forest.

Reptiles exhibited moderately heterogenous activity patterns relative to location. Sierra alligator lizards exhibited the strongest, and only, significant negative response to roadside habitat (0.41×, 0.22–0.74). Two species, the mountain garter snake and rubber boa, exhibited significantly higher activity in the forest in comparison to roadside habitat, while western fence lizard activity averaged highest under than ERS (Figure 6 and Table 3).

All measures of convergence and explanatory power indicated the model fit the data well. The community Bayesian *P*-value was 0.53, indicating the model had high explanatory power. Further, all species-level Bayesian *P*-values were between 0.1 and 0.9, except one (northern flying squirrel, $n = 11$, BP = 0.93).

4. Discussion

Wildlife crossing designs are known to play a critical role in how effective crossings are for different species. Results showed that the design of the ERS crossing has substantial potential to provide connectivity for a wide range of amphibian, reptile, and small mammal species. Across all species in our study, the results did not suggest species were avoiding the ERS crossing. All small animal species that were detected in the forest habitat were also detected under the ERS, with the exception of the broad-footed mole ($n = 1$) and northern flying-squirrel ($n = 11$). Amphibian activity generally trended highest in the forest habitat, while small mammals trended highest underneath the ERS structure. Estimates of activity for all amphibians, reptiles, and small mammals were lower in the more open roadside habitat, in comparison to the forest and under the ERS. These results suggest that adding cover objects, such as downed logs, and/or planting and maintenance of shrubs adjacent to the structure (but not to create access points to the top of the structure) may enhance passage for species that are more likely

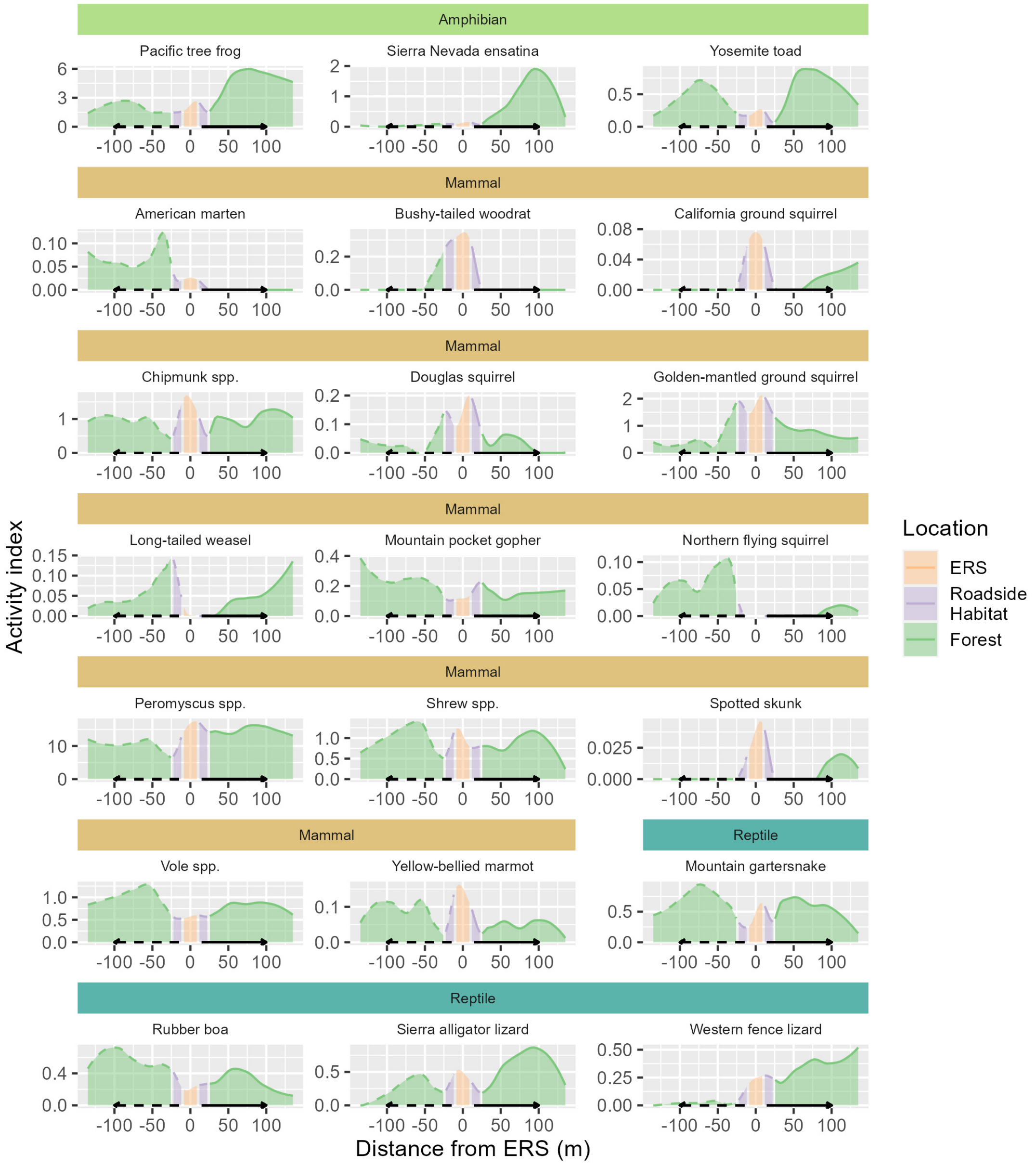

FIGURE 5
Spatial activity patterns of animals detected among specific camera locations within the elevated road segment (ERS), roadside habitat, and forest, for 21 species groups. ERS cameras are indicated by orange shading, roadside habitat cameras are shaded purple, and forest cameras are shaded green. Each species has a unique y-axis scale, for visual purposes. The *x*-axis represents the distance from ERS passage (0) in meters.

to avoid open areas (e.g., McDonald and St. Clair, 2004; D'Amico et al., 2015; Jackson et al., 2015). Only 9 small animals (4 frogs, 2 snakes, 2 lizards, and 1 mouse) were documented from weekly road mortality surveys within the project footprint over the 4-year period (S. Barnes, C. Vaughan, USFS, pers. com). Therefore, the evidence suggests the ERS design promotes permeability for small wildlife movement while also minimizing road mortality.

4.1. ERS Permeability: amphibians and reptiles

At the taxonomic group level, amphibian activity was significantly lower in the open roadside habitat compared to the interior forest, while mean activity was higher under the ERS structure than in roadside habitat (but less than forest). Roadside habitat was largely open without trees or shrubs and with little leaf litter in comparison to the interior forest. We postulate that these general trends in amphibian activity are most likely associated with the lack of leaf litter and cover in the roadside habitat. Amphibians other than Yosemite toads (e.g., Pacific tree frogs, *Ensatina salamanders*) were likely most often actively foraging within their home ranges, rather than migrating. Natural forest habitat with abundant leaf litter from trees and shrubs provides higher soil moisture levels for amphibians, reducing desiccation risk while also providing cover for their invertebrate prey (e.g., Duellman and Trueb, 1994). The ERS structure in the Sierra National Forest was also set on top of a solid impervious aggregate

TABLE 2 Community mean activity rate ratios (compared to ERS) and standard deviation parameters for random effects.

	Mean	SD	5%	95%
Community mean rate ratio				
Roadside habitat	0.57	0.10	0.41	0.72
Forest	0.69	0.19	0.39	0.98
Effort	1.46	0.24	1.07	1.83
Community standard deviation				
Intercept	2.00	0.36	1.40	2.50
Roadside habitat	0.35	0.21	0.00	0.63
Forest	1.10	0.26	0.68	1.50
Effort	0.54	0.17	0.26	0.80

The mean column corresponds to the mean estimate (rate ratio or standard deviation) for each parameter, the SD column corresponds to one standard deviation, and the 5 and 95% columns correspond to the lower and upper bounds of the 90% highest density interval. A rate ratio of 1.0 would be equivalent to activity under the ERS. There is a row in the community mean effects for the intercept parameter, as we included this as a non-centered random effect, with a fixed mean of 0.

roadbed, and we don't know if the lack of natural soil under the ERS affected passage use.

Similar to Pacific tree frogs and Ensatina salamanders, the relative mean activity of Yosemite toads under the ERS was higher than in the roadside habitat and lower than in the interior forest. Based on local expert knowledge and Yosemite toad telemetry (Liang, 2010), these toads were likely migrating from breeding meadow to upland habitat through the forest. Individual identification supported this, as individual toads were not documented along the fence on different days within the season, as would be expected if it was primarily used as foraging habitat (Brehme et al., 2022a). Although 2 toads were documented moving from one side of the fence line to the other without interacting with the ERS, 29 individual toads were documented moving under the ERS with no evidence of turning around after entering the passage (20 on HALT trigger, 9 on time-lapse), and we suspect many more toads passed under the crossing but were undetected. The fact that some toads reached but did not turn into the passage suggests that small segments of fencing perpendicular to the fence line and parallel to the passage (e.g., Langton and Clevenger, 2021) may be important to help change their trajectories and more effectively lead animals into the passage. However, a majority of unique Yosemite toads "gave-up" migration after moving back and forth along the extended forest fence lines; they presumably moved back to into the forest habitat (Brehme et al., 2022a). This may also help to explain the higher relative mean activity in the forest in comparison to the roadside for this migratory species and may further support the use

TABLE 3 Effect of location (under the ERS, roadside habitat, and forest) on the relative activity of amphibians, small mammals, and reptiles.

		ERS			Roadside			Forest		
		Mean	LCI	UCI	Mean	LCI	UCI	Mean	LCI	UCI
Amphibians	Pacific tree frog	4.21	2.38	7.45	3.29	2.00	5.43	6.13	4.40	8.55
	Sierra Nevada *ensatina*	0.33	0.16	0.68	0.20	0.10	0.42	0.55	0.34	0.91
	Yosemite toad	0.94	0.51	1.76	0.63	0.35	1.15	1.47	1.01	2.15
Mammals	American marten	0.13	0.05	0.34	0.06	0.02	0.18	0.13	0.07	0.24
	Broad-footed mole	0.02	0.01	0.11	0.01	0.00	0.07	0.01	0.00	0.05
	Bushy-tailed woodrat	0.77	0.4	1.51	0.39	0.20	0.79	0.05	0.02	0.12
	California ground squirrel	0.15	0.06	0.39	0.08	0.03	0.21	0.03	0.01	0.08
	Douglas squirrel	0.38	0.18	0.8	0.22	0.1	0.47	0.07	0.03	0.15
	Golden-mantled ground squirrel	7.56	4.61	12.38	4.76	2.97	7.64	2.12	1.51	2.96
	Lodgepole chipmunk	4.91	2.93	8.45	2.20	1.31	3.68	4.01	2.95	5.53
	Long-tailed weasel	0.17	0.07	0.43	0.10	0.04	0.25	0.18	0.1	0.33
	Mountain pocket gopher	0.68	0.35	1.32	0.38	0.19	0.73	0.75	0.49	1.11
	Northern flying squirrel	0.06	0.02	0.20	0.03	0.01	0.11	0.11	0.05	0.21
	Peromyscus mice spp.	71.3	43.8	114.0	35.8	23.2	55.6	51.2	38.8	67.6
	Shrew spp.	4.51	2.74	7.58	2.21	1.33	3.63	3.66	2.65	5.05
	Spotted skunk	0.09	0.03	0.26	0.04	0.01	0.14	0.02	0.01	0.06
	Vole spp.	2.35	1.33	4.05	1.58	0.94	2.64	3.34	2.43	4.66
	Yellow-bellied marmot	0.49	0.24	0.98	0.24	0.11	0.51	0.25	0.15	0.42
Reptiles	Mountain garter snake	1.52	0.88	2.72	0.93	0.54	1.64	1.83	1.29	2.62
	Rubber boa	1.31	0.73	2.44	0.92	0.52	1.63	1.75	1.22	2.53
	Sierra alligator lizard	2.18	1.24	3.86	0.91	0.50	1.69	1.59	1.09	2.24
	Western fence lizard	0.77	0.4	1.45	0.52	0.28	0.95	0.5	0.32	0.79

Mean model estimates and 90% confidence intervals are presented for 3 locations: (1) within elevated-road surface (ERS), (2) roadside, and (3) in forest.

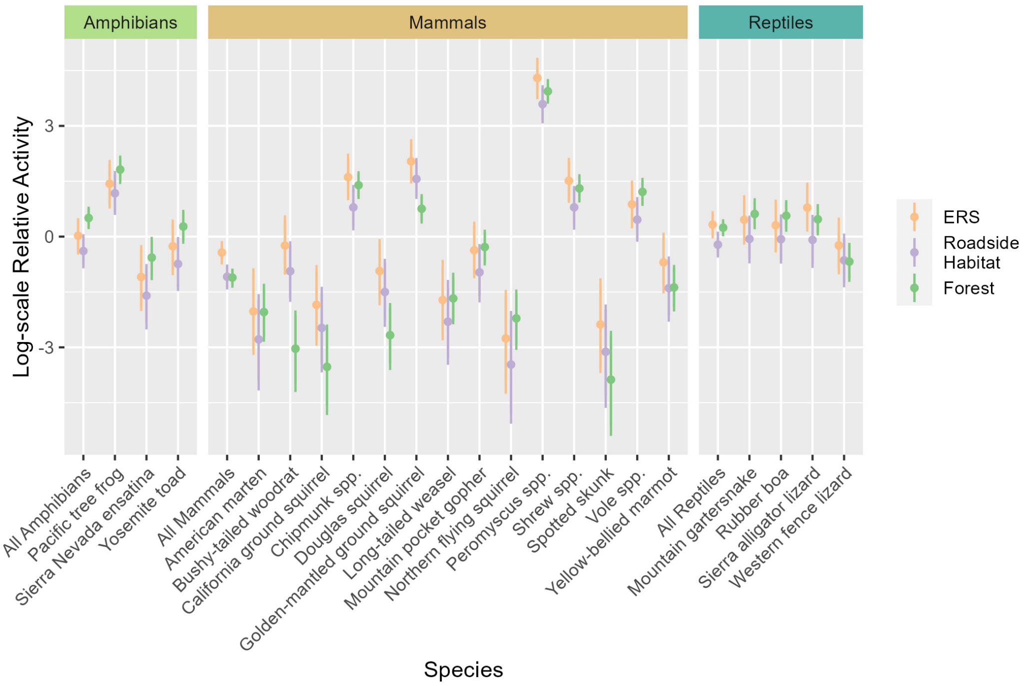

FIGURE 6

Marginal effect plot showing the difference in relative species activity among the elevated road segment (ERS), roadside habitat, and forest, for 21 species groups. Points represent the mean estimates for each species. Intervals represent the 90% highest density intervals (HDI), with thick lines represent the 75% HDI. Under ERS activity is represented by orange point-intervals, roadside activity is purple, and forest activity is green. The dashed line represents the community-wide average activity. Taxonomic mean effects are derived from the mean of model coefficients for species in that taxon and are represented by the intervals "all amphibians", "all mammals", and "all reptiles".

of mitigation designs with wide passages and/or frequent crossing opportunities for migrating amphibians.

Reptiles had similar patterns as amphibians, in that 3 out of 4 species (mountain garter snake, rubber boa, Sierra alligator lizard) had lower mean activity estimates in the open roadside habitat; however, mean activity for reptiles under the ERS and in the forest were almost identical. These species also prefer habitats with moisture and shelter opportunities, such as downed wood, logs, and leaf litter (Stebbins and McGinnis, 2012), which is consistent with lower activity in the open roadside habitat. Activity of western fence lizards, a habitat generalist, was similar between roadside and forest habitats and slightly higher under the ERS. These results indicate the ERS was also effective in promoting road permeability to the reptiles in our study site.

4.2. ERS permeability: small mammals

Many small mammal species are known to use road crossings (e.g., Clevenger et al., 2001; Martinig and Bélanger-Smith, 2016; Stewart et al., 2020), and our results support this for the ERS as well. All 14 small mammal species analyzed had higher mean estimates of activity under the elevated road in comparison to the roadside habitat, while 10 species also had equal or greater mean activity estimates under the ERS than in the forest. The ERS offers protection to most small mammals from aerial predators and medium to large carnivores. Therefore, it may be an additional source of refuge or shelter within the surrounding habitat, in addition to ground burrows or trees used for shelter by most small mammal species. No predation of small mammals or herpetofauna was observed under or adjacent to the passage during the study. It is also possible that many of these species may predate on invertebrates or other small vertebrate prey under the ERS. Although evidence of predator-prey interactions within road passages has been mixed (Mata et al., 2015; Caldwell and Klip, 2020; Martinig et al., 2020), in a recent study, mice and woodrats were observed using the safety of ledges and internal structural cover to prey upon invertebrates within large underpasses (Brehme et al., 2022b). Although we did not identify all invertebrates as part of this study, we did positively identify at least 104 occurrences of primarily larger beetles and spiders that set off the camera trigger under the ERS, indicating arthropods were also present under the ERS structure.

Three mammal species had almost no difference in mean activity between the ERS and roadside habitat with significantly higher mean estimates in the interior forest. These were the northern flying squirrel, mountain pocket gopher, and voles (California and/or Montane species). The northern flying squirrel is a forest obligate species that spends much of it time up in trees, while both the mountain pocket gopher and vole species are primarily subterranean, not venturing far from their burrow systems to forage above ground (Jameson and Peeters, 2004). Subterranean species in general may have been less likely to use the ERS, as they have increased sensitivity to degraded and impervious substrate and thus require suitable soils they can burrow under (Russell et al., 2007; D'Amico et al., 2015). We documented 28 detections of voles under the ERS during our study. Previous work in Banff National Park showed two species of specialist voles were absent from road-crossing structures, despite being abundant in connected forest and roadside grasslands (D'Amico et al., 2015). This may indicate the ERS is more permeable to vole movement than under-road culverts.

4.3. Limitations

The passage did present challenges in monitoring due to the wide monitoring area. Because we monitored along barrier fencing outside of the passage, we focused on monitoring the sides of the ERS closest to the barrier fencing that helped to lead animals from the forest to the passage. The width of the camera triggers covered a small proportion of the total width of the passage; therefore, we likely missed a substantial amount of activity from; (1) animals moving along the fence into the ERS that did not pass over a camera trigger and (2) animals that used the ERS passage without interacting with the adjacent barrier fencing. The camera triggers within the passage were up to 2.7 times the length of triggers within the habitat (1 m vs. 37 cm). This was important as many animals moving along the fence may not make a hard turn to enter the passage. The additional length should have helped to accommodate this, and some small bricks were set under the passage to help steer animals over the trigger without obstructing the openness of the passage. The height of the passage was also too low for efficient installation of cameras >1 m deep into the passage. Finally, we attempted to add cameras to the exits to document complete movements; however, with the number of available camera systems, we would have had to narrow the passages under the ERS to make this effective, which would have changed the attributes under the ERS to be closer to a small tunnel. Although we only effectively monitored activity under the west side entrances to the ERS, data from individual Yosemite toads did not show that any individuals that entered the ERS from the west side (over the 1 m trigger) subsequently turned around under the ERS (unpublished data, B. Ewing and C. Brehme, USGS). The HALT cameras with active infrared triggers used in this study have very high probabilities of detecting any animal that moves over the trigger (Hobbs and Brehme, 2017). Future monitoring would benefit from infrared trigger beams that span entire subsections of the passage. Also, advances are still needed in small affordable outdoor cameras to obtain high resolution, wide-angle night photos and videos at short focal lengths to capture the details of slow- and fast-moving small animals in remote settings, and there are some examples of other techniques that have been successful in smaller passages (e.g., Gleeson et al., 2018; Jarvis et al., 2019; Corva et al., 2022).

4.4. ERS designs for secondary roads, primary roads and highways

The prototype ERS in our study can theoretically be made to any length, creating a wide passage without constricting migratory movements to small tunnels (**Supplementary Data Sheet 1**; 2018 cost was approximately $40K USD). The prototype ERS also allows natural light, moisture and rainfall to permeate the length of the passage so that climate and moisture underneath is similar to that outside. The ERS system installed in the Sierra National Forest was built to meet USFS, City and County road specifications and can be removed and re-installed as desired (e.g., on a seasonal basis). This ERS has been in operation since 2018 with frequent use of off-road vehicles, large recreational vehicles, logging trucks, and fire trucks. Semi-annual to annual checks and maintenance have been required to ensure all bolts and connectors are tight. Replacement of wood mats or portions thereof may be required in the future. However, with ongoing regular maintenance, these may be permanent structures (Jon Fiutak, Anthony Composites, pers. com). Similar structures fabricated with other materials could also be designed.

For high-use primary roads and highways, we worked with engineers (Dokken engineering) to develop concept ERS designs that range from open low bridge type designs (pre-cast longitudinal bridge and pre-cast horizontal bridge) to less costly repeating passage designs along a raised roadbed (repeating elevated pre-cast box culverts or repeating short span pre-cast abutments: **Supplementary Data Sheet 2**). They include guardrails for traffic safety and can be designed to accommodate shoulders if necessary. Climbing barriers for small animals are also included along abutments and on the roadside walls between passages. Further information about these is provided in Brehme et al. (2022a). These engineered concept designs are meant to provide a starting point for local and DOT engineers to design and build permanent ERS type structure(s) to enhance the movement of migratory amphibians and other small wildlife species over wide stretches of high use roadways. They are planning level documents; therefore, additional engineering and reinforcements may be added to meet specific road requirements. For repeating passage designs, the distance between passages may be informed by fence movement distance studies of target or similar species, such as those previously conducted with common toads, California tiger salamanders, and Yosemite toads (Ottburg and van der Grift, 2019; Brehme et al., 2021, 2022a), or potentially informed by home range lengths for non-migratory species.

4.5. ERS and current passage design considerations

Overall, permeability of passages to small animal movements appear to vary greatly according to species behavior, location, passage spacing, and passage characteristics (e.g., Lesbarrères et al., 2004; Pagnucco et al., 2012; Beebee, 2013; Langton and Clevenger, 2017; Hedrick et al., 2019; Jarvis et al., 2019; Matos et al., 2019; Schmidt et al., 2020; Boyle et al., 2021). Recent evidence and literature reviews are starting to indicate that wider passages are generally more permeable to more small animal species than small tunnels (e.g., Martinig and Bélanger-Smith, 2016; Langton and Clevenger, 2017; Schmidt et al., 2020), although small tunnels have been shown to be effective for species in some locations, particularly where spacing between passages was short (i.e., Helldin and Petrovan, 2019; Jarvis et al., 2019). Finally, passages that provide microhabitat conditions (e.g., temperature, substrate, light, and vegetation) similar to the surrounding habitat have been shown to most effective [see reviews by Jackson et al. (2015), Langton and Clevenger (2017), and Gunson and Huijser (2019)].

In line with the current knowledge and guidance for small animal crossing systems, the ERS was designed so that target species can move along a relatively natural terrain path and cross the roadway with conditions similar to the surrounding habitat (Gunson and Huijser, 2019; IENE, 2020; Langton and Clevenger, 2021). All ERS passage designs (**Supplementary Data Sheets 1, 2**) have a suggested height of at least 0.3 m, and passage opening widths of 1.5 m wide or greater for repeating passage designs on a 2-lane roadway, to ensure better openness for high permeability to animal movement. Wider passages may be considered for wider multi-lane roadways. Except for ERS systems placed on top of roads, the designs incorporate natural soil bottoms. However, the "top of road" systems, like the prototype ERS in our study, allow for ease in installation and removal (e.g., seasonally) for lower use roadways. Grated openings on top of the ERS serve to decrease temperature and moisture differentials between the passage and outside environment, allowing some natural light within the passage, as well as moisture to reach the passage floor during rainfall events. These passage attributes are particularly important to help to minimize passage rejection for amphibians and reptiles, and potentially open-habitat specialist species (e.g., Jackson et al., 2015; Langton and Clevenger, 2017; Gunson and Huijser, 2019). This does not include having the passages used as road drainage systems, as this may result in inundation or increase pollutants that could potentially be harmful to amphibians (White et al., 2023). Also, noise from vehicles is not alleviated with these concepts and deserves consideration and study.

Finally, road barrier fencing is a common problem in the efficacy of passage systems due to the need for frequent maintenance (e.g., Baxter-Gilbert et al., 2015; IENE, 2020; Langton and Clevenger, 2021). In a recent review, 44 studies reported an issue with barriers within 2.2 years after installation due to gaps, washouts, deterioration and movement around, under, or over the fencing (Gunson and Huijser, 2019). There is some evidence solid fencing may be safer and more effective in leading small animals to passages than fencing they can see through (Milburn-Rodríguez et al., 2016; Brehme and Fisher, 2021; Brehme et al., 2022a, Conan et al., 2022); however, installation of solid fencing must be accompanied by consideration of potential effects on hydrological flows during rain events, particularly when rights of way are limited. The ERS designs eliminate or reduce the need for stand-alone barrier fencing along their length. All plans include smooth solid side walls and overhangs along bridge abutments or sides of raised roadbeds to prevent climbing and to keep small animals off the roadway, while barriers for traffic are included to meet safety standards for vehicles, bicyclists, and pedestrians. These designs do not require "jump-outs" to allow animals to escape back into the habitat if they wander onto the road, as do those that have elevated fencing (Langton and Clevenger, 2021). The ERS repeating passage design options for primary roads and highways are similar to systems in the Netherlands and elsewhere where topography, grading or lowered road verges on both sides of the road essentially create an elevated road surface bed, although here the passages are wide, and the footprint is limited to the road itself.

Although these designs may have a higher initial cost, there are other advantages in building elevated road structures in comparison to below grade crossings in some landscapes, including smaller area of impact, less susceptibility to flooding and inundation, and greater suitability in areas with challenging topography (flat lands, hilly, and extreme terrains).

5. Conclusion

Our analysis of the prototype ERS passage in Sierra National Forest, Fresno County, CA, USA, indicates that this wide and open design is permeable to movements of migratory and non-migratory amphibians, reptiles, and small mammals. Overall, amphibian and reptile activity under the ERS did not significantly differ from the forest, while small mammal activity was greater under the ERS than in the forest. Relative use of the ERS was equal to or greater than roadside habitat for all 22 species. Lower activity of some species in comparison to the interior forest can likely be attributed to decreased use of sparsely vegetated habitat within several meters of the road. These results suggest that adding downed logs and vegetation adjacent to the structure may enhance passage for these species.

Current road mitigation practices for small animals have primarily focused on amphibians and turtles, with designs largely comprised of stand-alone barrier fencing and small passages under the roadway. The breadth of scientific knowledge on the efficacy of these mitigation systems is limited but growing. Recent work has shown the negative filtering effect on amphibians if passages are too far apart or if fencing is too long. In general, the literature suggests that as a group, small animals are more likely to use and benefit from wider, more frequent, and more natural passages that offer cover or protection from larger predators. The ERS was designed to meet these needs and can theoretically be built to any length and width.

The ERS and additional concept designs provided are meant to increase the options available for consideration in road mitigation planning for small animals (**Supplementary Data Sheets 1, 2**), while incorporating the most recent scientific information. These and other possible ERS designs are particularly relevant for species or communities that require permeability across long lengths of roadway to help meet their connectivity needs. Although these designs were largely based on permeability to migratory amphibians, their use for non-migratory amphibians, reptiles and small mammals has been shown. They also offer alternative solutions for road mitigation systems in areas with challenging terrain and other hydrological or environmental constraints. We encourage the continued development and testing of these and other potential road mitigation solutions for small animals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for this study as passive camera methods were used for animal detection that did not involve capture, handling or marking of live vertebrates.

Author contributions

CB, SB, and JF designed the ERS passage. CB conceived the study with input from SB, MH, and RF. CV, SB, BE, and MH led the field effort for all camera data. BE analyzed all the photos, identified animals to species, and organized the database. PG performed the statistical analysis and produced results, figures, and tables. CB and PG wrote the manuscript. JF produced **Supplementary file 1**. CT, SH, and HS produced **Supplementary file 2**. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

Funding for the ERS and installation was provided by the U.S. Forest Service. Study funding was provided by the California Department of Transportation, Division of Research, Innovation and System Information (DRISI; Agreement 65A0553), Nevada Department of Transportation, Transportation Pooled Fund Program Project in 2022 (P342-20-803), and U.S. Forest Service, and Ecosystems Mission Area of the U.S. Geological Survey.

Acknowledgments

We thank the U.S. Forest Service (USFS) for funding the ERS and for conducting maintenance and camera monitoring in all years of the study. Sterling Walker and Jim Weber from Anthony Hardwood Composites/EMTEK worked with the authors to design the ERS and turn our vision into reality. ERTEC generously donated water-permeable polymer matrix fencing and materials. We thank USFS for assisting in the installation and maintenance of the fencing. It was a group effort including several of the authors and Devin Adsit-Morris, Denise Clark, Tristan Edgarian, Jennifer Kingston, and Chase Stafford (USGS); Wesley Burton, Tony Borelli, Alonso Ruiz, Mindy Mcclurg, and Phil Mazon (USFS); Ed Schiedel (Volunteer USFS). We greatly appreciate the work of Dokken Engineering for the ERS design concepts and guidance to include John Klemunes; and associated input and comments from Tom Langton (Herpetofauna Consultants International Ltd.), Carl Savage, Lindsay Vivian, Luz Quinnel, Robert Young, Robert Blizzard, Sophie Golding, Arnica McCarthy, and Blake Hirara (Caltrans). Finally, we also thank the California Department of Transportation, Division of Research, Innovation and System Information (DRISI; Agreement 65A0553) for helping to fund the initial stages of the project in 2018–2019, USFWS, and Hobbs Ecology for funding and support in 2020, and the Nevada Department of Transportation, Transportation Pooled Fund Program Project for funding in 2022 (P342-20-803). Partial funding was provided by Ecosystems Mission Area of the U.S. Geological Survey. This is Contribution Number 874 of the U.S. Geological Survey Amphibian Research and Monitoring Initiative (ARMI). Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Conflict of interest

CT, SH, and HS were paid to engineer concept designs for elevated road segments (see **Supplementary material**). JF designed (with USGS and USFS input) the elevated road segment installed in the Sierras and was paid for installation and materials. MH helped with the study design and was paid for camera rental.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer VP is currently organizing a Research Topic with CB and RF.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1145322/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 21 June 2023
DOI 10.3389/fevo.2023.971337

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RECEIVED 16 June 2022
ACCEPTED 25 May 2023
PUBLISHED 21 June 2023

CITATION
Hromada SJ, Esque TC, Vandergast AG, Drake KK, Chen F, Gottsacker B, Swart J and Nussear KE (2023) Linear and landscape disturbances alter Mojave desert tortoise movement behavior.
Front. Ecol. Evol. 11:971337.
doi: 10.3389/fevo.2023.971337

Linear and landscape disturbances alter Mojave desert tortoise movement behavior

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Introduction: Animal movements are influenced by landscape features; disturbances to the landscape can alter movements, dispersal, and ultimately connectivity among populations. Faster or longer movements adjacent to a localized disturbance or within disturbed areas could indicate reduced habitat quality whereas slower or shorter movements and reduced movements may indicate greater availability of resources. The Mojave desert tortoise (*Gopherus agassizii*) is a threatened species that is challenged by anthropogenic disturbances.

Methods: We studied tortoise movements using Global Positioning System (GPS) loggers at multiple sites in the Mojave Desert of Nevada and California. Tortoises at our sites encountered localized, linear human infrastructure, including paved roads, dirt roads, and fences, as well as landscape-scale disturbances [wildfire, off highway vehicle use (OHV), livestock grazing area]. We fit two-state (moving and encamped) Hidden Markov models to GPS logger data to infer how tortoise movement behavior relates to anthropogenic and natural features.

Results: We found that temporal covariates, individual-level random effects (intercepts), and sex best explained state transition probability in all sites. We compared relationships between tortoise movement and linear disturbances, which varied depending on site and context. Tortoises made longer movements within the OHV recreation area, near most dirt roads, and near a low-traffic paved road, indicating that tortoises avoid these habitat disturbances. Conversely, tortoises made shorter movements in areas of higher slope and near highways, suggesting that these features may restrict movement or provide resources that result in prolonged use (e.g., forage or drinking locations). Tortoises that encountered fences around utility-scale solar installations were more active and made longer movements near fences, indicative of pacing behavior.

Discussion: These results provide insight into how different disturbances alter tortoise movement behavior and modify tortoise habitat use, providing information that can be used to manage tortoise habitat.

KEYWORDS

roads, fencing, hidden Markov movement model, off-highway recreation, livestock grazing, wildfire

1. Introduction

Animal movement is a key component of behavioral and ecological processes such as foraging, social interactions, and dispersal (Hooten et al., 2017). Describing changes in movement behavior in response to environmental conditions can provide insight into the impacts of habitat disturbance for a given species. Technological advances in tracking devices provide detailed insight into animal movements, enabling a deeper understanding of how individual behaviors shape the ecology and conservation of species (Kays et al., 2015). The use of tracking devices provides the opportunity to evaluate conservation efforts such as translocation, delineation of movement corridors, as well the effects of anthropogenic disturbances on movement patterns (e.g., Zeller et al., 2012; Nickel et al., 2021; Picardi et al., 2022). Wildlife habitat continues to be transformed by anthropogenic disturbance and understanding the influence on animal movement can inform management strategies to ensure the persistence of at-risk populations. This can be especially true for wide ranging species that coexist with different disturbances in different contexts, where understanding how context interacts with a disturbance is important to developing management strategies.

The Mojave desert tortoise (*Gopherus agassizii*) is a medium-sized (generally <380 mm midline carapace length), terrestrial tortoise species native to deserts of the southwestern United States (Berry and Murph, 2019). The species is listed as threatened under the United States Endangered Species Act, as critically endangered by the International Union for Conservation of Nature, and has continued to decline since listing (U. S. Fish and Wildlife Service, 2011; Allison and McLuckie, 2018; Vaughn et al., 2020). Tortoise habitat has been altered by various anthropogenic disturbances, which range from local to landscape scales. Understanding how these disturbances alter the quality of tortoise habitat and restrict population connectivity are key recovery actions outlined in the recovery plan (U. S. Fish and Wildlife Service, 2011). Disturbance is generally thought to decrease habitat quality, but evidence used to infer how disturbance alters habitat quality and use is primarily limited to abundance estimates or counts of tortoise sign (Boarman and Sazaki, 2006; Tuma et al., 2016; Berry et al., 2020a,c), and not correlates of fitness or mechanisms that may be driving population declines. Prior research has indicated that anthropogenic disturbances alter tortoise movement, especially at local scales (Peaden et al., 2017; Gray et al., 2019; Hromada et al., 2020), but has left managers with a limited understanding of how both localized and landscape-scale disturbances may alter individual movement behavior. Describing the behavioral variation associated with disturbances can improve effective mitigation practices to protect populations from anthropogenic uses.

Transportation infrastructure, such as roads and railroads, is commonly located within tortoise habitat and can be a source of tortoise mortality as well as a barrier to tortoise movement (Boarman and Sazaki, 2006; Nafus et al., 2013; Rautsaw et al., 2018). Many areas contain networks of dirt roads, which can cause soil compaction and erosion, alter the physiology of native plants, allow for the incursion of predators, and restrict tortoise movement (Sharifi et al., 1997; Brooks and Lair, 2005; Gray et al., 2019; Hromada et al., 2020). Paved roads may attract tortoises by providing areas where water pools for drinking (Medica et al., 1980), and increased runoff can increase plant production in the surrounding margins (Lightfoot and Whitford, 1991). However, paved roads pose a higher mortality risk for tortoises and result in locally reduced tortoise densities (von Seckendorff Hoff and Marlow, 2002; Boarman and Sazaki, 2006; Nafus et al., 2013). To mitigate for tortoise mortality, many of the highways in tortoise habitat have been fenced to exclude tortoises. However, these fences can have unintended consequences, as tortoises will often pace alongside them attempting to access formerly used habitats and can consequently experience potentially detrimental thermal environments (Peaden et al., 2017; Nowakowski et al., 2020).

Other disturbances in remaining desert tortoise habitat include wildfire, grazing and off-highway vehicle (OHV) use. Wildfire, promoted by the spread of non-native Mediterranean grasses, has burned large areas of the desert southwest, often replacing perennial shrubland communities with annual grasslands (Esque et al., 2010; Brooks, 2012). Desert tortoises continue to move within these converted, post-fire landscapes, though loss of shrub cover may reduce thermoregulatory opportunities and consumption of non-native grasses may degrade tortoise health (Drake et al., 2015, 2016; Snyder et al., 2019). Sheep and cattle grazing continue to be important uses of public lands in the Mojave Desert, altering the vegetation community, increasing competition for forage, and damaging soils (Webb and Stielstra, 1979; Nicholson and Humphreys, 1980; Medica et al., 1982; Abella et al., 2019). Off-highway vehicle recreation has proliferated on public lands in the desert southwest, further spreading invasive plant species, and causing soil compaction/erosion, vegetation damage, and disturbance/mortality of animals (Ouren et al., 2007; Switalski, 2018). Prior research suggests that desert tortoise density is reduced or eliminated in areas of high OHV use, and that the closing of areas to OHV use benefits tortoise populations (Bury and Luckenbach, 2002; Custer et al., 2017; Berry et al., 2020b).

In this paper, we examine fine-scale tortoise movement data and relate movement behavior to environmental features. We studied tortoises that live alongside anthropogenic disturbances as well as in different habitat contexts (e.g., mountainous versus flat sandy terrain). Using a generalized Hidden Markov Model (HMM) framework, we demonstrate how disturbances alter both movement state and movement step length parameters. We expected to find strong relationships between tortoise movement behavior and natural features as well as anthropogenic disturbances.

Specifically, we hypothesized the following relationships between the environmental/disturbance covariates and tortoise movement behavior:

- Tortoises are more likely to be moving during the daylight hours and during spring, as this reflects their diurnal habits and known seasonal activity patterns.
- Male tortoises are more likely to be moving and make longer steps than female tortoises. This is expected due to known behavioral differences between tortoise sexes relative to space use and activity.
- When tortoises are near roads, they will be less likely to be moving, and if moving, will make longer step lengths when closer to roads. We expect this response as tortoises select for movement away from roads potentially due to low vegetative cover in roads.
- When tortoises are near linear barriers (fences and railways), they will be more likely to be moving state and will also make longer steps than when away from such features. We anticipate this as a

reflection of pacing behavior that tortoises often exhibit alongside barriers.

- When tortoises are moving through landscape-scale disturbances believed to degrade tortoise habitat (open OHV activity, grazing, wildfire), they will be more likely to be moving and make longer steps than in areas without these large-scale disturbances. We expect this due to the reduction of available resources and cover *via* degradation of tortoise habitat.
- When tortoises are in areas that have natural features that restrict movement and/or provide uncommon resources (areas of high slope and shrub cover), they will be more likely to be not moving and make shorter steps while moving than in areas that do not have these features.

2. Methods

2.1. Study site descriptions

We studied movements of adult tortoises in seven different locations throughout the Mojave Desert of Nevada and California (Figure 1) between 2011 and 2021. Sites primarily consisted of Mojave desert scrub dominated by creosote-bursage (*Larrea tridentata-Ambrosia dumosa*) associations, and other important shrub components (e.g., *Yucca* spp., *Psorothamnus* spp., *Cylindropuntia* spp.; Turner, 1994). Sites varied topographically from rugged mountain terrain in McCullough Pass, NV (max slope 38°) to flat sandy terrain at the Nipton site.

Collectively the sites were impacted by an array of disturbances including roads, railroads, solar development, OHV use, and recent wildfire (Table 1). Most sites had dirt roads used by recreational OHVs as well as power line maintenance traffic. The Silver State, NV and ISEGS South, CA sites had tortoise exclusion fencing surrounding utility-scale solar plants that were installed prior to data collection (2014 and 2011; respectively). The Nipton, CA site had a low-traffic volume paved road with no posted speed limit that served as access for an active railroad maintenance site that also intersected the study site. The China Lake, CA site had two unfenced, fairly high-traffic highways (speed limit = 88.5 km/h) that resulted in several tortoise mortalities in the years prior to and during our study (J. Hendrix, Naval Air Weapons Center—China Lake—pers. comm.). Portions of the surrounding public lands were designated for unrestricted "open" travel of OHVs; two areas were designated as seasonal grazing allotments for sheep in years of good forage production (Spangler Hills and Cantil Common; grazed 2019 and 2020 during our study period). The eastern portion of the site was within the off-limits boundaries of the China Lake Naval Weapons Station, which does not allow public access. The remaining areas in the China Lake site and all other sites were located on land managed by the United States Bureau of Land Management (BLM). The Hidden Valley, NV site experienced lightning caused wildfires during 2005 which led to large areas being

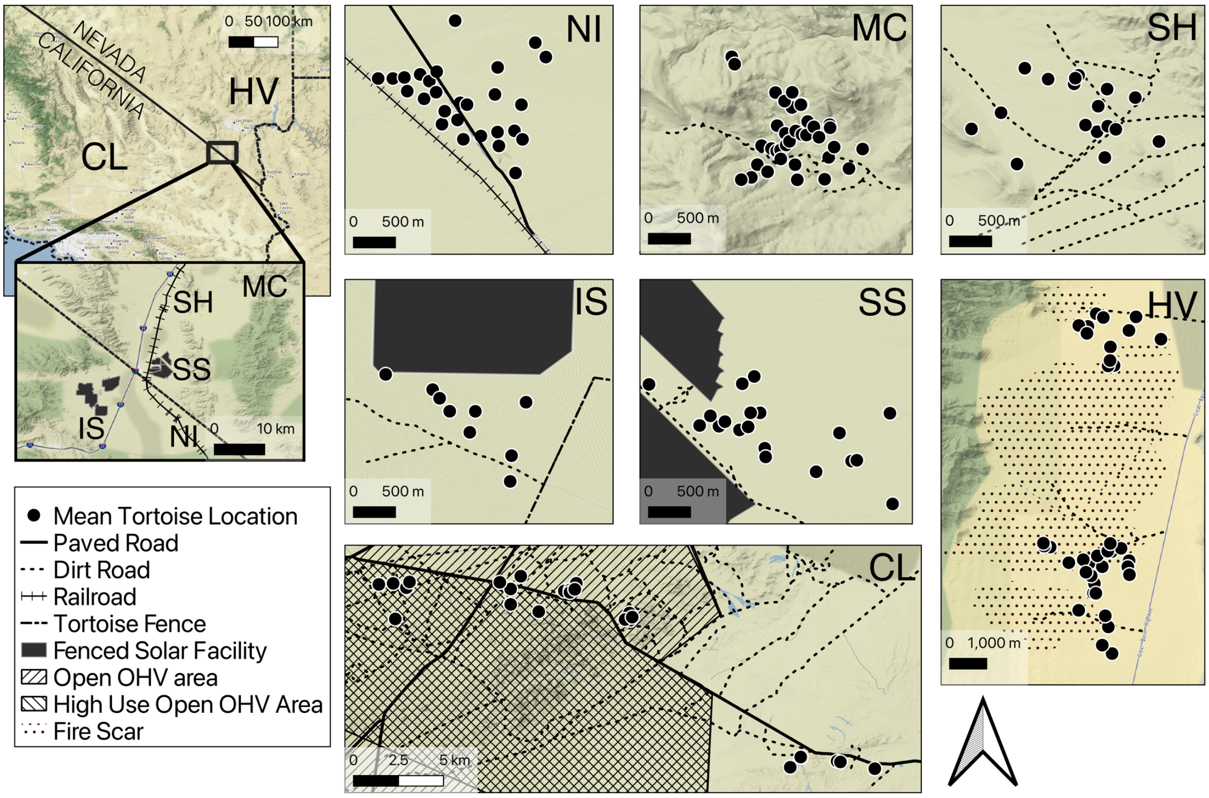

FIGURE 1
Overview of site locations and features within sites used to study tortoise movements in the Mojave Desert, CA and NV. Black dots represent the mean location for each tortoise with GPS logger data. Site codes: Nipton (NI), McCullough (MC), Sheep Mountain (SH), ISEGS South (IS), Silver State (SS), China Lake (CL), Hidden Valley (HV). Disturbance features are indicated in the legend. The edges of solar installations are fenced.

TABLE 1 Summary of tortoise global positioning system (GPS) telemetry data.

Site	Years	n Tortoises	GPS points	Topography	Disturbances
Nipton, CA	2019–2021	26	208,793	Flat, sandy	Paved road, railroad
Sheep Mountain, NV	2015–2021	17	127,660	Flat, with some hills	Dirt roads
McCullough Pass, NV	2015–2021	36	84,630	Rugged and rocky	Dirt roads
Silver State, NV	2016–2021	20	195,966	Deeply incised rocky washes	Dirt roads, fencing
ISEGS South, CA	2018–2021	9	66,142	Flat, cobble	Dirt roads, fencing
China Lake, CA	2019–2021	28	94,537	Flats and rocky hills	Highway, dirt roads,**open OHV area**,***grazing***
Hidden Valley, NV	2011–2013	19	13,194	Flat, gravel	Dirt roads,**wildfire scar**
Total		155	790,922		

Bold indicates landscape-scale disturbances that were used as categorical predictors in models, all other disturbances were considered as continuous covariates. All localized anthropogenic disturbances were log-transformed. See text for specifics regarding the OHV area in China Lake and the burned area in Hidden Valley.

burned (Drake et al., 2015). Site characteristics are summarized (Table 1) and maps of sites are provided (Figure 1).

Tortoises were found during surveys of one-km^2 plots at Nipton, McCullough Pass, Sheep Mountain, ISEGS South, and Silver State as part of another study (Mitchell et al., 2021). Surveys were also conducted in the area within 1 km of Randsburg Wash Road (China Lake) and on 400 m^2 vegetation study plots in Hidden Valley (Drake et al., 2015).

2.2. Tortoise telemetry protocol

All tortoises involved in the study were outfitted with Very High Frequency (VHF; Model RI-2B; Holohil Systems Ltd.) radio-transmitters. Tortoises at all sites except Hidden Valley were equipped with GPS loggers (i-gotU GT-120; Mobile Action Technology). Tortoises at these sites were physically relocated once per month using VHF telemetry equipment. During these visits the GPS loggers were swapped for battery recharge and data downloading. Tortoises at the Hidden Valley site were outfitted with custom-made Advanced Telemetry Systems GPS/VHF combination loggers. Tortoises at the Hidden Valley site were relocated at approximately one-week intervals, and GPS loggers were replaced approximately every 3 months for data downloading and charging. All tortoises were greater than 200 mm midline carapace length; tortoises smaller than this size could not carry both the radio and logger without excessively modifying the vertical profile of the tortoise. Most GPS loggers were programmed to collect a point every hour, but some of the Hidden Valley and McCullough Pass data were collected every half hour during initial deployment trials and later filtered to provide an hourly dataset. The number of tortoises monitored, and GPS locations recorded per site are provided in Table 1. Thirteen of the twenty tortoises at the Silver State site were translocated from the footprint of the nearby utility-scale solar installation in 2014, and one of the nine tortoises at the ISEGS South site was translocated from the nearby utility-scale solar installation in 2011. Tortoises were translocated to areas just outside the footprint of the utility-scale solar installations. All but one of the tortoises that interacted with the fences were translocated. Therefore, we were not able to separate movement behavior differences due to the fences and translocation; translocation is known to alter movement behavior in tortoises (Nussear et al., 2012). All tortoises were handled in accordance with a USFWS Permit (permit TE-030659-10), Nevada Department of Wildlife Scientific Collection Permit 317351, University of Nevada, Reno Animal Care and Use Committee protocol (IACUP 00671), and a Memorandum of Understanding with the California Department of Fish and Wildlife (all to T. Esque).

2.3. Environmental predictors

We chose environmental predictors that we hypothesized were important to tortoise movement behavior and used them as covariates in our model construction. We used the TIGER road datasets for dirt and paved roads in our study areas and modified them to better match satellite imagery (United States Census Bureau, 2021). We log-transformed distance to linear features (roads, railroad, and fences) to reflect the spatial decay around the impact of these features as we expected the effect of these covariates to be localized near the disturbance. We used the National Land Cover Dataset (NLCD) shrub coverage layer to determine if differences in shrub cover may act as a cue for tortoises to recognize disturbed areas (Homer and Fry, 2012; Rigge et al., 2021). To represent areas burned in wildfire, we digitized a polygon that corresponded with the burned areas seen on Google satellite imagery as available layers from the BLM did not accurately reflect the wildfire boundaries visible on satellite imagery and from data collected while tracking tortoises (Drake et al., 2015). Additionally, we considered slope as it has been shown to alter tortoise movement (Hromada et al., 2020); slope was derived from the USGS Digital Elevation Model using the terrain function in package raster (U.S. Geological Survey, 2017; Hijmans et al., 2022).

Open OHV areas on BLM lands typically have both established routes designated by the BLM and user-created routes that are often missing from BLM road and trail inventories. BLM designated open routes are intended for travel by street legal vehicles, while user-created routes are tracks created by OHVs driving outside of established routes. We found that established routes in the Spangler Hills OHV Area at the China Lake site were best represented by data sourced from Owlshead GPS, a service intended for OHV recreationists (Friends of Jawbone, 2022). We also used a polygon from the same source to represent the entire open OHV area. However, we found that although the entire area is designated for off-trail OHV use, certain areas within the boundaries used by our tortoises saw little to no off-trail use, likely due to their greater distance from major staging areas, or rougher terrain. These included the areas north of Randsburg Wash Road and areas that were rocky hills with large boulders. We created another polygon that excluded these areas and tested both as categorical predictors in our modeling efforts. Sheep grazing occurred during years of higher productivity across the area contained within the entire OHV polygon; thus, the two polygons represent a combination of grazing pressure and relatively high OHV use versus mostly just grazing pressure with relatively low OHV use.

2.4. Discrete-time hidden Markov models

Hidden Markov models (HMMs) are a popular approach for analyzing temporally regular animal location data for inference on movement behavior (McClintock et al., 2012; McClintock and Michelot, 2018). HMMs are a form of state-space models that assume that observed data arise from a number of "hidden" (i.e., latent) states. In the case of telemetry data these are movement behavior states that can represent activities such as exploration, foraging or rest (Patterson et al., 2009). The state changes can be identified from differences in the distribution of the step lengths and turning angles of the relocation dataset (Morales et al., 2004; McClintock et al., 2012). Many HMMs fit to animal movement data can only identify two biologically meaningful states; one that corresponds to a moving state and one that corresponds to a resting or encamped state (Beyer et al., 2013; McClintock et al., 2014). An extension of the HMM framework (known as a generalized HMM) allows for inference of changes in behavioral states and movement parameters using environmental and individual level covariates (McClintock et al., 2017; Carter et al., 2020).

We fit generalized discrete-time hidden Markov movement models (HMMs) to tortoise GPS location data using the package momentuHMM (version 1.5.4) in program R (version 4.0.4; McClintock and Michelot, 2018; R Core Team, 2018). Hidden Markov movement models require bursts of continuous GPS data; however, tortoise behavior (i.e., burrowing and cave dwelling) often results in missed fixes. As we are primarily interested in above-ground activity in this study, we were not concerned about missed fixes during burrow use. We partitioned our GPS telemetry data first to contain temporally continuous segments that had no more than 2 h of missing data and greater than 36 consecutive hours. To filter out inaccurate fixes from the GPS loggers, we used the uere.fit function from package ctmm to estimate error for the GPS loggers based on locations taken with stationary loggers placed at study sites (Calabrese et al., 2016). We placed GPS loggers within unoccupied tortoise burrows to determine how this positioning would alter fix accuracy. For the custom made ATS GPS loggers (used in the Hidden Valley site), we fit an error model using the HDOP (Horizontal Dilution of Precision) data column as a predictor of horizontal error to the stationary logger data. For the i-gotU GPS loggers, we fit an error model without using the EHPE (Expected Horizontal Position Error) column as much of the data were retrieved from the loggers without the column. We filtered extreme outlier points for the i-gotU GPS loggers by removing points from both the stationary logger and tortoise telemetry datasets that had greater than 25 m of elevation error recorded altitude—elevation from a digital elevation model (Laver et al., 2015), and then fit an error model on the stationary logger data. We then used the outlie function in ctmm to detect and remove telemetry points that had a movement rate greater than that expected for a tortoise (300 m/h; Nussear and Esque, unpublished data) after accounting for locational error.

As HMMs assume exact temporal consistency, GPS data had to be imputed by fitting a continuous-time correlated random walk to the data using the crawlWrap function from the momentuHMM package as a wrapper around the crwlMLE function from package crwl (version 2.2.1; Johnson and London, 2018). We fit 2-state HMM models with the two states representing a "moving" state and an "encamped" state. The "moving" state was described by longer hourly step lengths and low turning angles while the "encamped" state was described by short hourly step lengths and high turning angles. We fit a two-state model as we anticipated that our data would not allow for differentiation between different tortoise activities when movement lengths were below 10 m (e.g., foraging vs. thermoregulating) and different movement purposes (e.g., nesting vs. socializing; Pohle et al., 2017). We used a gamma distribution for the step length and a von Mises distribution for turning angles as well as a zero-mass parameter to account for time steps with no movement.

We first fit models to determine if environmental covariates altered the probability of state transition between the moving and stationary states. Tortoises are largely diurnal, and their activity is largely determined by environmental conditions such as temperature and food availability which change throughout the year (Woodbury and Hardy, 1948; Ruby et al., 1994b; Rautenstrauch et al., 2002; Ennen et al., 2012). We fit models incorporating hour and Julian date and their interaction to find the best combination of the two variables to explain transition probability of tortoises changing from encamped to moving states. We used the cosinor function from momentuHMM on the hour variable to allow state transition probability to reflect the typical diurnal activity patterns of desert tortoise (Zimmerman et al., 1994). We also tested models that looked for relationships between sex and transition probability, as male tortoises are often more active than females (Agha et al., 2015a). We then used the top-ranked ranked model (based on AIC), representing circadian and circannual movement patterns, to test additional environmental predictors that we hypothesized to alter the transition probabilities of movement states.

We fit generalized HMMs to the best performing state transition model that related lengths of hourly time steps to environmental covariates and the sex covariate. We restricted our modeling of step related covariates to only consider effects of environmental covariates on steps made while in the moving state, as the mean step length in the encamped state was below that of the positional error (~ 10 m) of our datasets. We fit models relating both the mean and standard deviation of step length and the mean and concentration of turning angles to the environmental covariates. We compared models with additive combinations of step covariates using AIC to find the most supported combination of covariates. For the Hidden Valley site, we examined the potential for an interaction term between the categorical burn predictor and distance to dirt roads. We fit and compared models for each site separately as not all anthropogenic features were present at each site, and we wanted to determine if covariates had similar effects among sites. We also attempted to fit random intercepts for each tortoise as a covariate in both the transition and step models to allow for the potential of individual-level differences in parameters.

3. Results

Tracking of 155 different tortoises across seven sites resulted in 790922 GPS locations after filtering (Table 1). After filtering data from both logger types, the resulting dataset had hourly relocations with an estimated location error of 10 meters. Stationary GPS loggers within tortoise burrows failed to record any fixes during a month of deployment, indicating that tortoises located in burrows would be unlikely to have GPS locations recorded. We successfully fit 2-state HMMs to our data from each site. The moving state was characterized by longer hourly step lengths and more narrow turning angles, while the encamped state was characterized by short hourly step lengths and wider turning angles (Figure 2). Mean hourly step lengths for the moving state differed moderately amongst sites.

3.1. State transition models

The top-ranked behavioral state probability models were similar across our seven study sites, although the best models for some sites did not include all covariates tested, as presented in Table 2. AIC tables for models run at each site are given in the Supplementary Materials. All top models performed better than null models with no covariates. We found that an interaction between the continuous day-of-year variable with the cyclical hour variable best accounted for state, where tortoises were more likely to be moving in the earlier days of the active season (spring) and more likely to be moving during the daylight hours (Figure 3). The best model for every site included sex as a predictor, with males more likely to be in the moving state and more likely to remain in the moving state once moving (Figure 3).

State transition probabilities were affected by distance to dirt roads at every site except Hidden Valley, though the AIC difference was low between the best model at that site and the model including the dirt road covariate (Δ2.4 AIC). In all sites with distance to dirt road in the transition model, tortoises were more likely to be in the moving state when close to a dirt road, except for China Lake and Silver State which showed the opposite relationship (Figure 4). Distance to paved road was included in the top transition model for the Nipton site, but was not in the top model for China Lake, although the difference in AIC was relatively low between the best model and the model that included the paved road covariate for this site (Δ1.1 AIC). Tortoises at the Nipton site were more likely to be moving when closer to the paved road. The distance to railroad covariate was not included in the top model for the Nipton site; likely because tortoises were seldom located close to the railroad. Distance to fence was included in the top model for both sites where fences were present and indicated that tortoises were more likely to be moving when close to the fence (Figure 4).

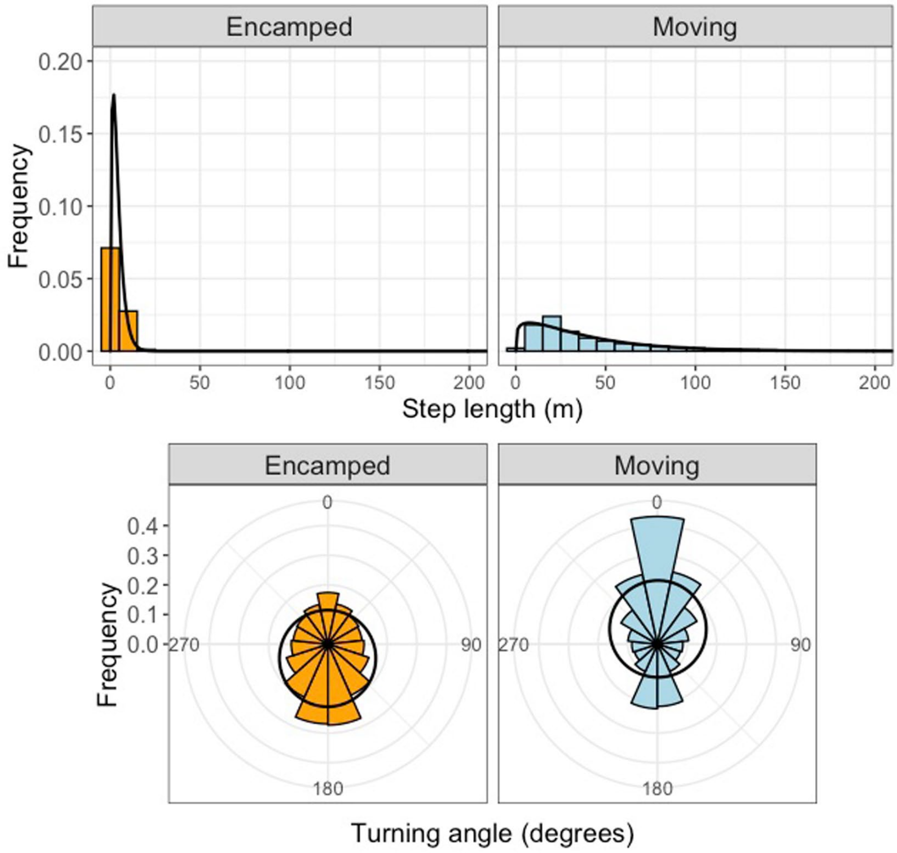

FIGURE 2
Step length and turning angle distributions for different movement behavior states for models fit to Mojave desert tortoise movement data. The encamped state (orange) was characterized by shorter mean step lengths and high turning angles while the moving state (blue) was characterized by longer mean step lengths and low turning angles.

The categorical covariate representing the OHV use area was in the top model for China Lake; tortoises were more likely to be moving in areas with relatively low OHV use and grazing than in areas without these disturbances. Distance inside the burn scar was in the top transition model for the Hidden Valley site, suggesting tortoises were more likely to be moving in areas closer to the edge of the burned area (Figure 5). Slope was included only in the best transition model for the Sheep Mountain site, suggesting that tortoises are more likely to be in the moving state in areas of higher slope. The only site that contained the shrub covariate in the top model was Nipton, which suggests that tortoises were more likely to be moving in areas of higher shrub cover. For all sites, a random intercept model for each individual was found to improve the AIC as there was considerable variation amongst individuals (Supplemental Figure 1). However, we chose not to include this in subsequent model fits as it added considerably to computational times and did not provide additional information on the covariates of interest.

3.2. Step length models

All top-ranked step length models performed better than null models; selection tables of all models fit examined are available in the Supplemental Materials. Dirt roads were included in the top model for every site where present. Hourly step lengths were both longer and more variable when close to dirt roads at every site except Silver State (Figure 4). The covariate for distance to paved road was in the top step parameter model for both the Nipton and China Lake sites, though they had the opposite effects. When moving closer to the paved road in Nipton, tortoises had longer hourly steps. In contrast, tortoises at China Lake had shorter hourly steps when closer to the paved road (Figure 4). Distance to fence was included as a covariate in the step length models for both the Silver State and ISEGS South sites, which were the only sites with fences. Hourly steps were longer when tortoises were moving near the fences (Figure 4). The OHV covariate representing the relatively heavy use area was included in the step model for China Lake, indicating that tortoises took longer

TABLE 2 Covariates that were in the tested in hidden Markov movement models for tortoise movement data.

Site	**Paved road**, railroad,**shrub, sex,**	**Paved road,** railroad, **shrub**,**sex**
Nipton, CA	**Dirt roads**, shrub,**slope, sex**	**Dirt roads,** shrub**, slope, sex**
Sheep Mountain, NV	**Dirt roads**, shrub, slope,**sex**	**Dirt roads, shrub, slope, sex**
McCullough Pass, NV	**Dirt roads**,**fence**, shrub, slope**, sex**	**Dirt roads, fence,** shrub,**sex**
Silver State, NV	**Dirt roads**, fence, shrub**, sex**	**Dirt roads, fence,** shrub
ISEGS South, CA	**Dirt roads**,**highway**,**entire OHV area**, shrub, slope**, sex**	**dirt roads, highway, slope,** shrub,**slope, high-use OHV, sex**
China Lake, CA	Dirt roads, shrub,**distance inside burn, sex**	**dirt roads x burn scar**, shrub,**sex**
Hidden Valley, NV	**Paved road**, railroad,**shrub, sex,**	**Paved road,** railroad**, shrub**,**sex**

Covariate in each cell represent covariates considered for models at each site, bold covariates indicate a covariate was in the top AIC ranked model for that site.

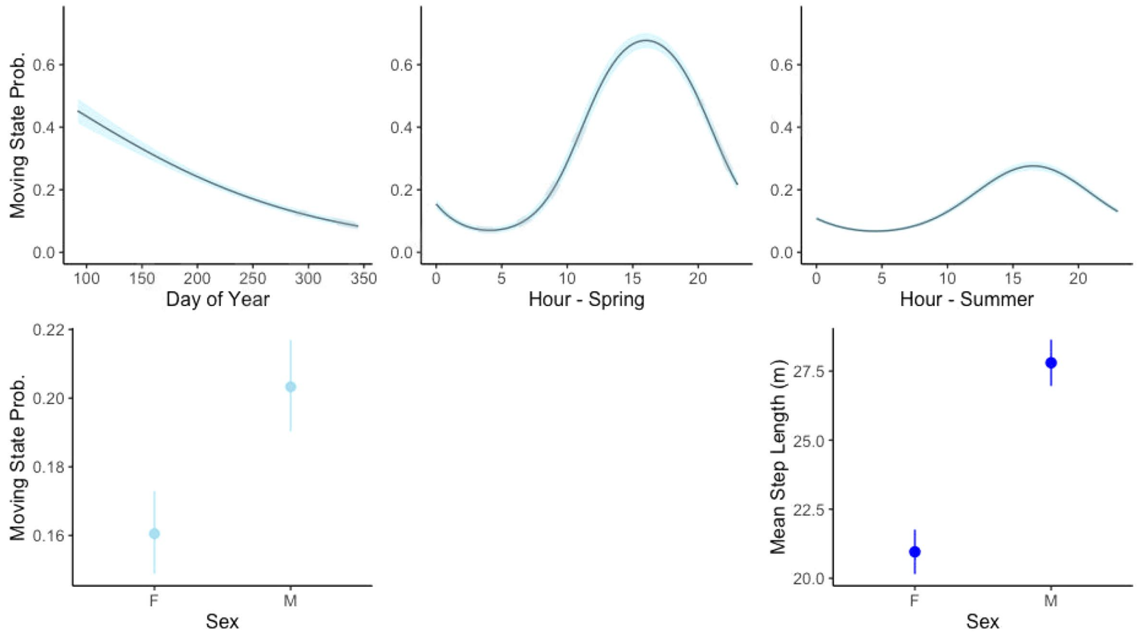

FIGURE 3
Relationship between covariates between stationary state probability (movement behavior state with other covariates held constant) and mean step length in the moving state for variables included in all models of tortoise movement.

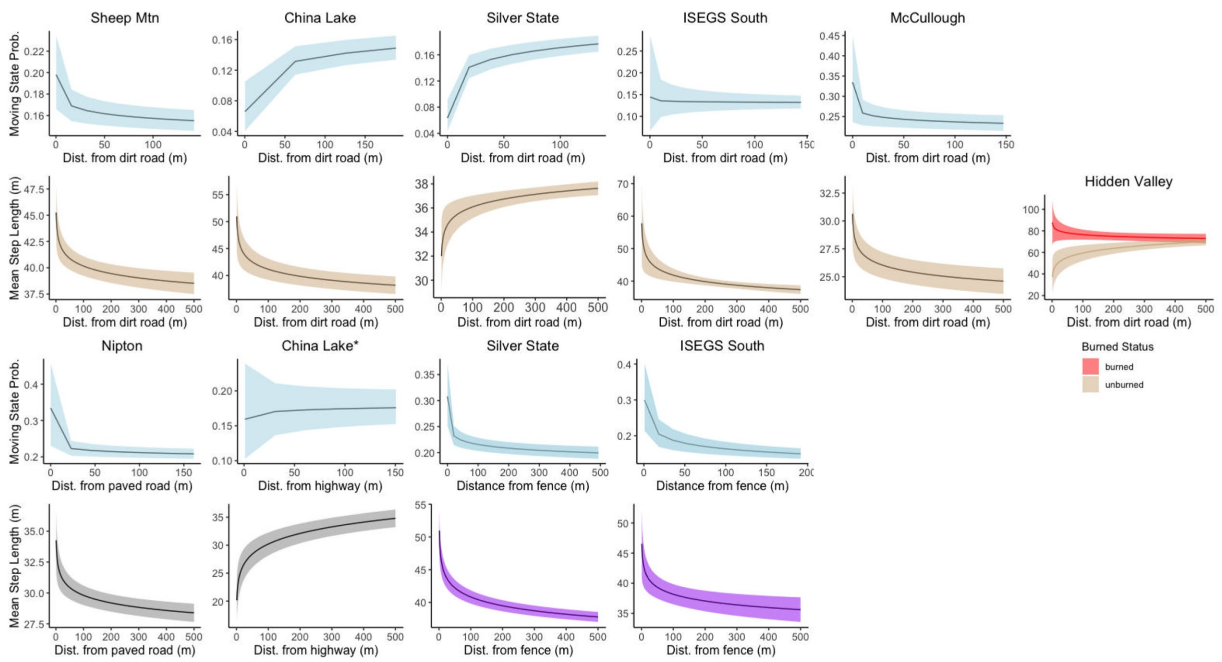

FIGURE 4

Stationary state transition probabilities and movement step lengths for covariates related to linear disturbances at different sites for tortoise movement. Tortoises responded to disturbances differently in different contexts. *The relationship between movement state and distance to highway was not in the top model for China Lake but is displayed for informational purposes.

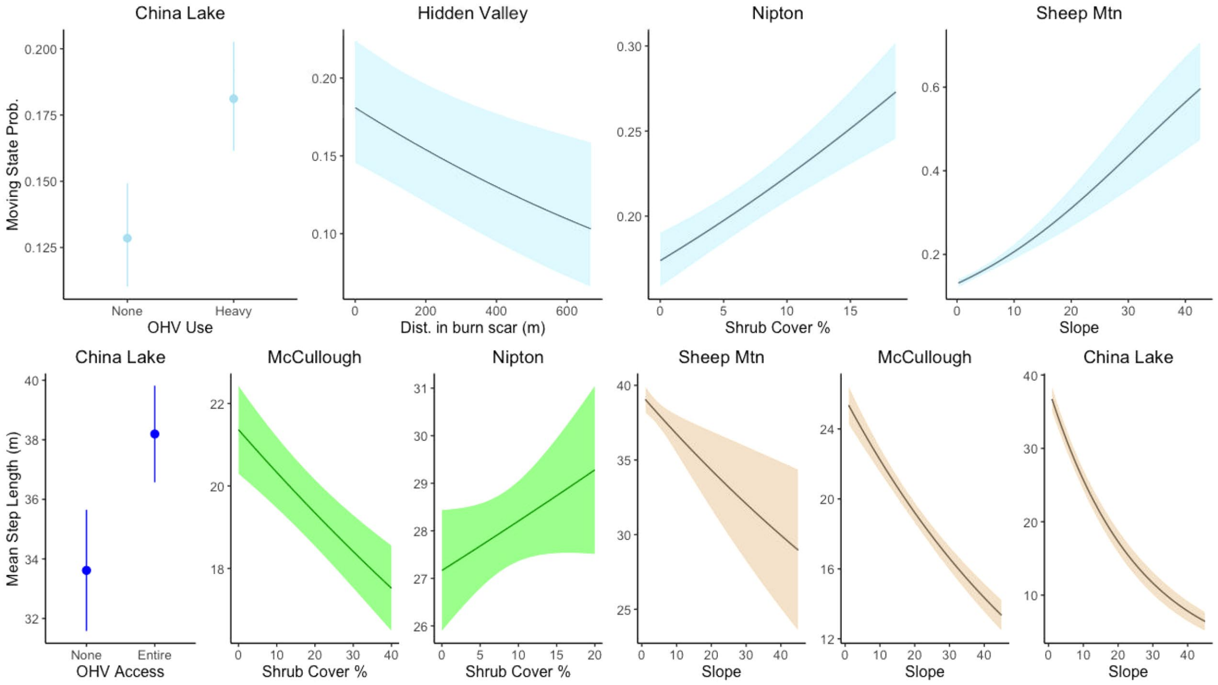

FIGURE 5

Stationary state transition probabilities and movement step lengths for covariates related to natural habitat features and landscape scale disturbances at different sites for tortoise movement. Tortoises responded to disturbances differently in different contexts.

hourly steps when moving in the high OHV use area (Figure 5). The fire scar covariate was included in the best model for the Hidden Valley Site with an interaction with the road covariate that indicated that tortoises took longer hourly steps near roads in burned areas but shorter hourly steps near roads in unburned areas (Figure 4). All the sites that had varied terrain (McCullough Pass, China Lake, Sheep

Mountain) included slope in the top step model, indicating that tortoises took shorter steps in areas of higher slope (Figure 5). The shrub covariate was in the best model for McCullough pass and Nipton and indicated that hourly steps were shorter in areas that had higher shrub cover for McCullough pass, but hourly steps were longer in areas of high shrub cover for Nipton (Figure 5).

4. Discussion

Describing changes in animal movement behavior in relation to environmental conditions is an important step toward understanding how disturbances and habitat features may shape ecological processes for a species. Our results describe fine-scale tortoise movement behavior that corroborates prior knowledge, and importantly, provide insight on movement responses to anthropogenic disturbances. As hypothesized, known relationships between temporal period and tortoise sex were recovered by our models. Tortoise behavior is known to change from day to night and throughout the year, and our models captured the diurnal habits of the species, as well as reduced movement propensity in hot summer months (Rautenstrauch et al., 2002; Inman et al., 2009). Our models also found that male tortoises are more likely to be moving and make longer movements than female tortoises, similar to the findings of other telemetry-based studies (Agha et al., 2015a; Sadoti et al., 2017). Tortoises typically move less in years of lower forage production, although they make longer movements when doing so (U. S. Fish and Wildlife Service, 1994; Duda et al., 1999; Sadoti et al., 2017). Increased movements likely occur in areas with reduced resources due to disturbance, especially as tortoises seek out rare forage items on the landscape (Jennings and Berry, 2015). In this study, our models consistently recovered relevant parameters confirming preexisting knowledge of tortoise ecology, and we demonstrated that HMMs provide a suitable method to analyze movement behavior in the presence of variable conditions. In addition, we found that tortoises typically responded to disturbances as we hypothesized, at both linear and habitat scales, though that these responses can depend on different contexts.

4.1. Natural habitat features

We found that tortoise movement behavior is influenced by natural features such as slope and shrub cover. Tortoise movement is limited by high slope (Hromada et al., 2020), and we found that tortoises made shorter hourly movements in higher slope areas at all three sites with varied terrain. This finding could be due to the higher cost of movement through rougher terrain, or potentially higher availability of resources in rugged areas. Sloped areas in tortoise habitat often feature rock outcrops that can provide areas where water pools during rain, shelter sites, forage cover, and areas of higher thermal inertia that may buffer extreme temperatures (Nowicki et al., 2019). The only site where we recovered a relationship between movement state and slope is also the only site on which tortoises moved between creosote flats and hills. Some studies have suggested that Mojave desert tortoise populations in areas with varied terrain may be more resilient to declines than those in flat areas (Allison and McLuckie, 2018; Berry et al., 2020c). Future research should focus on the causes and potential importance of these areas in preserving the species. We found that shrub cover was an important predictor of tortoise movement at only two of the seven sites in our study (Nipton and McCullough Pass). At Nipton, we found that tortoises were more likely to move, and take longer hourly steps, in areas of higher shrub cover, while tortoises at McCullough Pass were more likely to take shorter hourly steps in areas of higher shrub cover. The Nipton area is flat, sandy, and has relatively low shrub cover; areas of higher shrub cover generally occur along washes where tortoises prefer to move, though foraging opportunities may be lower (Jennings and Berry, 2015; Gray et al., 2019; Hromada et al., 2020). In contrast, McCullough pass is rugged and rocky with much higher overall shrub cover; tortoises may move more slowly in areas of higher shrub cover here due to higher availability of desirable resources (e.g., shade, forage underneath shrubs), and higher slopes often associated with that cover. While previous studies have shown that tortoises prefer moving in areas of higher perennial vegetation cover (Sadoti et al., 2017; Hromada et al., 2020), our results illustrate the importance of site-specific context in how tortoises are responding to environmental conditions.

4.2. Responses To linear and habitat disturbances

4.2.1. Paved roads

Our results indicate that tortoises respond differently to roads in different environmental contexts. Heavily trafficked paved roads have been associated with localized tortoise decline and shown to fragment tortoise populations (Nafus et al., 2013; Dutcher et al., 2020). Tortoises at the China Lake site took shorter steps when near the high-traffic highways, and distance to the highways did not appreciably affect the probability of being in a moving behavioral state. One possible reason for this could be that these tortoises perceive the danger of the highway and make shorter movements in an attempt to cross at a safe time, or they may become startled by traffic and withdraw into their shells as an attempted defense mechanism. Turtles, and many other species of reptiles, are considered 'pausers' in their response to traffic (Jacobson et al., 2016). Desert tortoises are known to respond by pausing to perceived threats, which may lead to shorter hourly step lengths near roads with high traffic levels (Ruby and Niblick, 1994). Some of the paths tortoises took near the highway ran parallel to the highway and may represent foraging trips taking advantage of increased plant growth from the road run off, or just a reluctance to cross the highway (Brooks and Lair, 2005). Although all tortoises monitored at China Lake ($n = 28$) were found within 1 km of the highway, only four (including one dispersing juvenile too small for a GPS logger) ever crossed the highway. While this could be due to road avoidance, it is also possible that the established home ranges of these animals did not include the highways. Tortoise mortalities were noted on the China Lake highway in years prior to and during our study period; thus, the remaining tortoises that continue to cross may be the few individuals that have so far managed to survive crossing. Our findings at China Lake may also be related to how the few crossings we observed. The tortoises that crossed the highway spent little time near the road when crossing, so paths of other tortoises along the road would likely have overwhelmed the small sample size of crossing events captured by our data. The one male tortoise that made multiple crossings of the highways at China Lake lost its GPS logger after two crossings, so data for those events were not available for our analysis.

We found the opposite relationship with the low-traffic paved road in the Nipton site, which may represent less perceived risk. When tortoises choose to make a movement near it, they may make the movement faster than they would otherwise, as the road typically provides no resources outside of rain events that pool water on the road surface (Medica et al., 1980). One additional difference between the highway at China Lake and the paved road at Nipton is that the soft shoulders of the China Lake highways are actively maintained (graded) while the Nipton road shoulder has infrequent maintenance. This additional disturbance of the soil around the highway may cause tortoises to alter their behavior, as the soil is less compacted (potentially more difficult to move through), but also may provide forage plants (e.g., *Astragalus* sp.) that are not commonly available elsewhere and may attract tortoise activity (Jennings and Berry, 2015).

4.2.2. Dirt roads

Our results indicate that dirt roads typically influence tortoise movement behavior in the way we hypothesized, but not always in the same manner. Prior research has demonstrated that tortoises are less likely to make movements in the vicinity of dirt roads (Sadoti et al., 2017; Gray et al., 2019; Hromada et al., 2020), yet tortoises still encounter and cross these disturbances regularly. Our results suggested that tortoises were more likely to be in a moving state closer to roads in some sites (McCullough Pass and Sheep Mountain), while they were more likely to be in the encamped state closer to dirt roads in others (China Lake and Silver State). This may be due to the different context of the roads at these different sites; the areas where the roads exist in China Lake and Silver State are more disturbed by anthropogenic activities. Many of the dirt roads at China Lake are located within sandy washes where OHV traffic has resulted in loose soil that may discourage tortoise movement. The main road at Silver State falls within a rocky wash in the area and is located ~40 m from the fenced solar facility; and many of the translocated tortoises use burrows and shelter sites directly adjacent to the road. The pattern recovered from our other sites, where tortoises are more likely to be moving when near a road, suggests that where dirt roads are in more undisturbed contexts, tortoises are more likely to be moving when near them. We found that when tortoises are moving near roads, at all sites aside from Silver State, they were more likely to make longer hourly movements. Tortoises have been noted to make movements along gravel roads (Grandmaison et al., 2010; Agha et al., 2015b), and it might be the case that roads allow for faster movement through some areas. However, as tortoises are known to avoid moving near roads, this behavior may be indicative of avoidance behavior, in that tortoises could be moving faster to minimize the time they spend in an exposed areas that offer few resources and increased exposure to threats. Movements of tortoises that interact with roads at Silver State are restricted by fences and they are forced to spend more time closer to roads. The primary road at this location is in a wash and used as part of an annual OHV race. As tortoises cannot as easily move away from the area directly affected by the roads, they may move slower to use resources associated with the wash, and to avoid perceived danger from road activity. These differences in tortoise movement behavior are influenced by different contexts of roads and may play a role in connectivity of tortoise populations.

4.2.3. Fencing

Fences have been built to prevent desert tortoises from accessing high-traffic highways throughout its range, and fenced utility scale solar energy development has been constructed on previously suitable habitat. As these fences often intersect areas that were previously available to tortoises, individuals will often pace the fence attempting to return to previously known resources (Ruby et al., 1994a; Peaden et al., 2017; Sadoti et al., 2017). Tortoises are more likely to be moving in and took longer hourly steps in areas closer to the fences supporting observations of these prior studies, and as we had hypothesized. Vegetation was removed from areas immediately adjacent to fences at our study sites; thus, tortoises moving along the fences are likely exposed to dangerous thermal environments that may influence movement behavior (Peaden et al., 2017; Nowakowski et al., 2020). Tortoises that interacted with fences were primarily translocated tortoises, with only one exception. This non-translocated tortoise, originally found outside the solar footprint in the ISEGS South site, continued to pace along the fence 10 years after its construction, and the translocated tortoises at the Silver State site continued to pace the fence 7 years post construction. This suggests that these linear disturbances can have a long-lasting impact on the space use and movement behavior when placed into formerly occupied tortoise habitat and used to exclude former resident tortoises. Translocated tortoises are known to move more than resident animals when translocated into unfamiliar habitat (Nussear et al., 2012); the increased movement behavior and movement length near the fence are similar to these prior findings. There may be a different behavioral response to fences by tortoises that were not subject to fence construction within their established home range area (e.g., offspring of tortoises translocated from solar installations or tortoises that disperse into an area with a fence). Studying animals that encountered fences throughout their lives would help us to better understand the potential long-term effects that fences may have on tortoise populations.

4.2.4. Off-highway vehicle activity and grazing

Off-highway vehicle recreation continues to grow in popularity within the deserts of the southwestern United States, as desert urban growth continues to sprawl, and off-highway vehicles become more capable and accessible. We were unable to independently estimate the effects of livestock grazing and OHV use on tortoise movement at China Lake, due to multiple simultaneous land uses. However, we found that the covariates representing different levels of this OHV disturbance, indicated tortoise movement behavior was expressed in different ways. The probability of being in a moving state was higher in areas that had both OHV and grazing activity, suggesting that tortoises may need to move more often and more quickly due to scarcity of resources, and potentially in recognition of dangerous conditions. Research on movement in other species has shown context dependent relationships between movement behavior and human land use. African wild dogs moved more often and faster in areas of lower resources but slowed when human activity was present (Creel et al., 2020). Indeed, movements by a wide variety of species are influenced by anthropogenic habitat disturbance and human presence (Doherty et al., 2021). Both OHV activity and livestock grazing reduce plant cover, damage tortoise burrows, and cause tortoise mortalities (Lovich, 1999; Switalski, 2018). The best model supported longer hourly step lengths in the high use OHV area designated area. This could be due to a variety of factors. Tortoises may be influenced by high OHV use areas in a similar manner as they are with dirt roads, making faster movements to avoid areas with low resources and unsuitable environmental conditions. Many of the routes created by OHV activity in the high-use area are in sandy washes, creating potentially difficult moving conditions for tortoises and destroying vegetation important for both thermoregulation and forage. Prior work has shown that tortoise sign is reduced in areas with active OHV recreation in washes (Custer et al., 2017). As we noted in the results for the Nipton site, tortoises may be using washes as movement areas, and some of the signal that we found may be from the use of the washes by both tortoises and OHV recreationalists for movement. Livestock grazing in the Mojave Desert may have some effects on plant cover and soil conditions. Grazing has been shown to cause soil compaction as well as a reduction in cover of native annual and perennial plants, increasing the potential of colonization by invasive annual plants (Webb and Stielstra, 1979; Nicholson and Humphreys, 1980; Brooks et al., 2006). Sheep grazing occurred throughout the OHV area. The BLM is tasked with assessing rangeland health of areas leased for grazing, yet the two allotments in our China Lake study area (Spangler Hills and Cantil Common) have never been assessed (Public Employees for Environmental Responsibility, 2022). The adjacent allotment (Rudnick Common) did not meet the BLM's standard of rangeland health when last assessed in 2004 (Public Employees for Environmental Responsibility, 2022). The alteration of tortoise movement caused by the compounded effects of both landscape scale disturbances (i.e., OHV use and grazing) provides further evidence that tortoise habitat is degraded in these situations. More research would help us better understand how these separate land uses effect tortoise movement and populations.

4.2.5. Burned habitat

Wildfire is a relatively new disturbance on desert ecosystems. Historically, fire was limited in the Mojave Desert and rarely spread to landscape scales; however, the establishment of non-native, annual grasses has allowed for fire to rapidly spread between shrubs and effect large areas of suitable tortoise habitat (Brooks and Esque, 2002; Brooks and Matchett, 2006). Desert tortoises continue to inhabit burned areas, and health and reproductive output does not seem to be affected (Lovich et al., 2011, 2018; Drake et al., 2015). However the invasive annual grasses that typically colonize burned areas have been shown to have negative impacts on health and survivorship in juvenile tortoises (Drake et al., 2016). Our data came from an area that burned six years prior to GPS telemetry collection. VHF telemetry data from these animals suggested that tortoise use of the burned areas had declined by this time, concurrent with the decline of *Sphaeralcea ambigua*, an important cover and forage plant, in the burned area (Drake et al., 2015). Our results suggested that the tortoises were less likely to be moving in areas deeper in the fire scar, though lack of GPS logger data from the years immediately after the fire precluded any interpretation of how tortoise movement changed as time since fire increased. We also found that tortoises interacted with dirt roads differently depending on whether the roads were inside the fire scar or not; suggesting that tortoises make longer steps near roads in the fire scar and shorter steps near roads when not inside the fire scar.

4.3. Limitations

Although our analysis leveraged a large GPS dataset, we were limited in scope to only making inference on tortoise surface activity, as our GPS units could not record data while the tortoises were underground. Thus, our inference is on the surface activity of tortoises as data on time spent inactive in burrows was not available for this analysis. Although tortoises spend most of their time in burrows (Rautenstrauch et al., 2002), we were mostly concerned with how tortoises are moving. However, understanding where tortoises use burrows in relation to disturbances is also important in describing how disturbances alter tortoise space use. The use of an additional data loggers, such as a light-sensitive loggers, would enable researchers to capture the times that a tortoise was inactive and in a burrow. This could allow additional inference on surface activity and on how different disturbances and environmental settings may influence this. However, we have demonstrated the utility of these methods to describe the movement behavior of a reptile and how it is influenced by disturbed habitat from different sources. Though outside the scope of this study, the addition of site and year specific weather covariates that may also influence tortoise movement (Duda et al., 1999) would allow for a more comprehensive model of tortoise movement behavior.

Individual-level variation in behavior can be an important aspect to consider in movement ecology (Merrick and Koprowski, 2017; Spiegel et al., 2017). Tortoises have distinct personalities that can play an important role in determining movement patterns and responses to stressors (Currylow et al., 2017; Germano et al., 2017; Le Balle et al., 2021). Animal personality potentially determines how an individual responds to an environmental disturbance or features (Michelangeli et al., 2022), and it is likely that tortoises with different personalities have different propensities to cross roads or pace fences. If so, tortoises that are more likely to cross a road are more susceptible to mortality. As a result, the distribution of the variation in personality syndromes in a population may be altered, which could have emergent effects on population function (Spiegel et al., 2017). Although our results indicated that there was considerable individual-level variability around movement state probability, these models took considerable computational time and models with random slope terms typically failed to converge. Further exploration of individual-level movement variation and personality is warranted to better understand how personality in this species may influence spatial distribution of tortoises and the implications for genetic connectivity.

4.4. Management implications

Our results indicate that tortoise movement behavior is altered by both local and landscape scale disturbances. Both the initial, and the revised recovery plan for the Mojave desert tortoise recommends non-essential or redundant routes to be closed (U. S. Fish and Wildlife Service, 1994, 2011), and our results suggest that this action could aid in restoring historic connectivity of tortoises populations. Although fences alter tortoise movement behavior, they continue to be important tools in restricting tortoise access to dangerous areas; further work could better describe how mitigation (e.g., shade structures) may alter how tortoises interact with fences. OHV activity and grazing continue to be prevalent uses of public lands in the southwestern US; our results suggest that these uses may be degrading tortoise habitat. Further study of how these disturbances affect tortoise populations is warranted to better understand how areas designated for these purposes influence landscape scale connectivity and viability of tortoise populations.

Data availability statement

The datasets presented in this article are not readily available due to the sensitivity of the species and easily accessible study sites. Requests to access the datasets should be directed to: stevehromada@gmail.com.

Ethics statement

The animal study was reviewed and approved by University of Nevada, Reno Animal Care and Use Committee protocol.

Author contributions

SH assisted in data collection, analyzed data, and wrote the manuscript. KN, TE, AV, and KD designed the overall projects, conducted and supervised data collection, and provided substantial comments on the manuscript. FC, BG, and JS managed field logistics and database upkeep and consistency as well as provided substantial comments on the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the U.S. Bureau of Land Management, the U.S. Fish and Wildlife Service, the National Fish and Wildlife Foundation (8006.15.058835 and 8006.20.070838), Clark County Desert Conservation Program, USGS Priority Ecosystem Science Program, Coyote Springs Investment Corporation, the U.S. Geological Survey Ecosystems Mission Area Energy and Wildlife Program, and the China Lake Naval Air Weapons Station Environmental Management Division. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Acknowledgments

We thank the many people who collected tortoise telemetry and other associated data, especially R. Inman, P. Medica, A. Modlin, A. Macdonald, B. Cunningham, S. Murray, G. Berry, E. Gaylord, G. Gantz, R. Van Gaalen, S. Lewicki, S. Dahl, B. Smith, S. Thomson, *C. Silva*, C. Quick, A. Poage, M. Walden, K. Vittum, C. Phillips, S. Snyder, B. Jacobs, M. Walden, G. Olson, V. Van Zerr, R. Kunicki, A. Terry, J. Willand, E. Sinnott, A. Chaney, D. Essary, T. Carmona, B. Reynolds, J. Jay, R. Lamkin, A. Berger, M. Kang, P. Cosmann, P. Emblidge, C. Aiello, R. Saulino, R. Lamkin, K. Bauer, A. Smith, S.Steel, P. Van Linn, K. Huxster, M. Poelman, B. Waitman and others. We are particularly grateful to Amy Fesnock (BLM—California), Mark Slaughter (BLM—Nevada), and Julie Hendrix (China Lake NAWS) for their support of our desert tortoise research program. We also thank K. Dutcher, A. Parandhaman, C. Carroll, and W. Plafcan for helpful comments on an earlier version of the manuscript. We thank B. Prochazka and two peer reviewers for their constructive reviews of this manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.971337/full#supplementary-material

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frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 25 September 2023
DOI 10.3389/fevo.2023.1059461

OPEN ACCESS

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RECEIVED 01 October 2022
ACCEPTED 17 August 2023
PUBLISHED 25 September 2023

CITATION
Sisson GP and Roosenburg WM (2023)
The rocks are hotter on the other side
of the fence: roadside habitats should
inform mitigation design.
Front. Ecol. Evol. 11:1059461.
doi: 10.3389/fevo.2023.1059461

The rocks are hotter on the other side of the fence: roadside habitats should inform mitigation design

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Maintaining viable populations of large reptiles is often challenging in road fragmented landscapes. While mitigation structures can reduce impacts, few studies have investigated how mitigation success can be affected by roadside habitats. In southeast Ohio, USA, we evaluated mitigation effectiveness for state-endangered timber rattlesnakes (*Crotalus horridus*) at a new highway in a forested landscape. Road construction at the study site created a wide corridor of open canopy habitats (the right-of-way; ROW) containing roadcuts and stone piles. However, exclusion fencing was constructed along the forest-ROW boundary, leaving the open canopy habitats on the road-side of the fence. Over three years, we monitored 6 rattlesnakes using radiotelemetry and found that rattlesnakes repeatedly crossed the fence to access forest-edge and ROW habitats. Rattlesnakes ostensibly crossed through damaged sections of the fence. The ROW was used most intensively by gravid females (n = 2), with their core home ranges overlapping the ROW by more than 50 percent. Despite the fence crossings, all home ranges were bounded by the highway and no rattlesnake road mortality was observed. Operative temperature models revealed that the ROW provided warmer thermal regimes that were rare or unavailable in the forest. On average, field preferred gestation temperatures (T_b = 29.7°C, SD = 1.8) could be attained or exceeded for more than 5 times as many hours per day in the ROW (7.8 hours) than in the forest (1.4 hours). Habitat selection models indicated gravid females selected warmer thermal habitats that were spatially concentrated in the ROW and edge habitats, while non-gravid snakes avoided the ROW beyond the forest edge. Habitat use within the ROW was mostly limited to rocky microhabitat structures, especially riprap stone piles and subsurface rock crevices on roadcuts, which provided buffered thermal regimes with refugia from extreme temperatures during the day and warmer T_e through the night. In forested landscapes, we encourage road planners to consider whether new road corridors are likely to introduce basking sites, and if so, maintain those features on the habitat-side of exclusion fencing, and consider restoring basking sites in the surrounding forest to reduce the potential for ecological trap formation.

KEYWORDS

road ecology, landscape, heterogeneity, reptile, timber rattlesnake, thermal biology, resource selection

1 Introduction

Road fragmented landscapes are becoming increasingly ubiquitous, with at least 25 million kilometers of new roads anticipated by 2050, representing a 60% increase to the global road network (Laurance et al., 2014). The continued expansion of the global road network stresses many wildlife populations through direct effects on survivorship and indirect effects resulting from landscape change (Forman and Alexander, 1998; Watson et al., 2018). Roads commonly impact wildlife populations through wildlife-vehicle collisions, population fragmentation, and resource loss and isolation (Forman and Alexander, 1998; Forman et al., 2003; Marsh and Jaeger, 2015). While modified and fragmented landscapes fail to meet traditional definitions of wilderness, they often retain some native biodiversity and are proving to be more valuable in landscape conservation strategies than once thought (Neilly et al., 2016; Wintle et al., 2018; Phillips et al., 2020; New et al., 2021).

The conservation value of road fragmented landscapes is likely enhanced when paired with effective mitigation (Clevenger et al., 2001; Dodd et al., 2004; Jackson et al., 2015). To sustain wildlife populations in road fragmented landscapes, best management practices often recommend exclusion fencing and crossing structures as means of reducing wildlife-vehicle collisions and maintaining ecological connectivity (Rajvanshi et al., 2001; Huijser et al., 2008; Clevenger and Huijser, 2011; Gunson et al., 2016; Boyle et al., 2021). These mitigation designs often consider the landscape context from the perspective of eliminating mortality hotspots and protecting animal movement corridors (e.g., Gunson et al., 2012).

An often-overlooked consideration of mitigation design is how roadside habitats, often created and maintained as part of the right-of-way (ROW), function within landscapes and affect mitigation success. We consider ROWs generically as human-made linear corridors maintained to service transportation infrastructure or utilities, including roads, gas pipelines, and electrical transmission lines. These corridors are often maintained through vegetation control, especially the removal of overstory trees. Road construction typically results in the loss, fragmentation, and modification of habitats, but in certain landscapes the road ROW may generate novel heterogeneity that serves as functional habitat or even refuge in an inhospitable matrix (Clevenger and Huijser, 2011; Kasten et al., 2016).

By altering the availability and configuration of habitats, roads can affect animal behaviors, such as resource selection and movement (Sartorius et al., 1999; Klingenböck et al., 2000; Berger, 2007; Andrews et al., 2008; Abrahms et al., 2016). One of the most common resources altered by roads is the thermal environment. In forested landscapes, vegetation is often controlled along roadsides, maintaining open canopy corridors of early successional vegetation and edge habitats exposed to increased insolation (Trombulak and Frissell, 2000; Forman et al., 2003; Harper et al., 2005; Kearney et al., 2009). In mountainous regions, the geophysical environment is often altered through earth-moving to create passes at reduced grades, leaving exposed rock escarpments adjacent to the road at modified slopes and aspects (roadcuts), and thus altering heat load (Sears et al., 2011). Stone piles (riprap) are sometimes dumped along the roadside to direct drainage and control erosion, but also function as buffered microhabitats that can serve as heat sinks (Huey et al., 1989). These thermal resources maintained in road corridors and other ROWs are sometimes used or preferred by heliothermic ectotherms, including forest-dwelling reptiles that require basking sites and or thermally exposed nesting habitat (Sartorius et al., 1999; Klingenböck et al., 2000; Blouin-Demers and Weatherhead, 2001; Shine et al., 2002). Because thermal resources can directly affect physiology and behavior, especially in ectotherms (Huey, 1982; Huey, 1991; Angilleta, 2009), such roadside habitats have the potential to benefit wildlife populations when these resources are otherwise scarce on the landscape.

However, when roadside habitats attract animals to the proximity of roads, use of those resources can come at the cost of increased wildlife-vehicle collisions or mortality from other causes (Langen et al., 2015). From this follows three important considerations: 1) if roadside areas provide habitat resources selected by wildlife, they could function as either compensatory habitats or ecological traps depending upon the cost of use (Jackson et al., 2015); 2) efficacy of road mitigation structures, particularly exclusion fencing, may be affected by roadside habitat composition and configuration relative to the landscape; and 3) effective placement of mitigation structures may allow would-be traps to function as viable habitat. In summary, if road corridors can provide habitat resources and the primary cost of using those habitats is road mortality, then it may be important for mitigation structures to allow access to roadside habitats while preventing access to the roadway itself.

One species that exemplifies roadside habitat tradeoffs is the timber rattlesnake (=*Crotalus horridus*). Timber rattlesnakes are large forest-dwelling snakes native to eastern North America and reported to be declining by the IUCN Red List. While numerous studies report negative demographic and genetic responses to roads (Rudolph et al., 1999; Clark et al., 2010; Bushar et al., 2015), other researchers have reported timber rattlesnakes to use roadcuts and other open canopy habitats found along roadsides, particularly by gravid (pregnant) individuals (Brown, 1993; Reinert and Zappalorti, 1998; Anderson, 2010). The species shows stark intraspecific variation in habitat preferences, ostensibly due to their foraging and reproductive strategies that are seemingly made exclusive by thermal constraints (Gardner-Santana and Beaupre, 2009). Timber rattlesnakes are ambush predators, and often remain sedentary for multiple days while waiting for prey (Reinert et al., 1984). This foraging strategy may drive preference for forested habitats with high canopy closure where thermoconforming will not result in exceeding thermal tolerances, forcing individuals to abandon an ambush site or expend excess energy metabolically. In contrast, gravid females maintain precise and elevated body temperatures that facilitate embryonic development (Gardner-Santana and Beaupre, 2009). Throughout pregnancy, gravid females often forgo foraging and show preference for canopy gaps that feature rock or woody microhabitat cover, providing basking sites with refuge where individuals can actively thermoregulate (Reinert, 1984; Herr et al., 2020). Thus, it would be expected that creation of an open canopy ROW could affect conspecifics differently by generating gestation habitat at the cost of foraging habitat. Landscape heterogeneity introduced by ROWs could thus be important to populations where basking sites are sparsely distributed across the landscape. However, because the species' life history is also characterized by delayed maturity, high adult survival, and infrequent reproduction (Ernst and Ernst, 2003), use of these roadside habitats could easily compromise population viability if it results in additive mortality.

Few studies have investigated how road mitigation design and roadside habitats interact to affect animal behavior and mitigation performance in snakes (Macpherson et al., 2021). We evaluated the effects of a mitigated highway on timber rattlesnakes in southeast Ohio, where the species is listed as Endangered under State law (Ohio Revised Code 1531.25). We report how a mitigated highway affected movement and space use by rattlesnakes, and how thermal resources impacted mitigation success as a function of exclusion fence design and placement. We predicted that thermal conditions preferred by gravid females would be available for more hours of the day in the ROW and edge habitats compared with the surrounding forest, and that preferred gestation habitats would be concentrated within the ROW. We also predicted that surface habitats within the ROW would exceed voluntary thermal tolerances, and that non-gravid snakes would avoid those habitats.

2 Materials and methods

2.1 Study site

We studied timber rattlesnakes in the Wayne National Forest (WNF) in Ohio, USA at the Nelsonville Bypass (NVBP). The WNF comprises 271 km^2 of non-contiguous forestland distributed across southeastern Ohio. The NVBP is a high speed (112 km/h), high volume (17,000 vehicles/day), four-lane divided highway (13.6 km length) that was constructed in WNF between 2007 and 2013, and was opened to traffic in October, 2013 (for a map of the study site, see Supplementary Figure 1). The woodland along the NVBP is an Oak-Hickory (*Quercus-Carya*) deciduous forest with rolling hills that feature sandstone outcroppings that are mostly shaded by overstory trees. The NVBP fragmented the forest landscape and created a large ROW (approximately 250 ha) of predominantly open canopy habitats. We define the NVBP ROW as the modified habitat area created during road construction, and not the paved road itself. Along the NVBP, the width of the ROW between the road and the forest ranged from 5–150 m on either side of the highway. The ROW habitats included road cuts of exposed sandstone, early successional stands of black locust (*Robinia pseudoacacia*) and sumac (*Rhus* sp.), fields dominated by mixed grasses and weedy vegetation (Poaceae, Asteraceae), barren slopes with exposed soils, and large riprap stone piles installed for erosion and drainage control (for photos of these habitats, see Supplementary Figures 2, 3).

Timber rattlesnakes were known to occur in this part of WNF based on incidental encounters reported to the Ohio Department of Natural Resources, but little was known about the population prior to construction of the NVBP. Pre-construction surveys (200 person hours) performed by local wildlife consultants failed to detect timber rattlesnakes in the project area, but two individuals were discovered within the ROW during construction. The Ohio Department of Transportation (ODOT) installed rattlesnake mitigation structures along 1.6 km of the NVBP in the area where rattlesnakes were encountered (described below). While parts of our research spanned the entire 13.6 km length of the NVBP, we focused most of our rattlesnake field work around the 1.6 km section of highway that was mitigated for rattlesnakes, and the adjacent ROW and forest habitats.

2.2 Mitigation design

To prevent deer and other large wildlife from entering the roadway, ODOT constructed a 2.4 m tall exclusion fence ("wildlife fence") along the entire 13.6 km length of NVBP. The wildlife fence was installed along the ROW-forest boundary (i.e., at the forest edge), and thus ranged from 5–150 m away from the road. However, the mesh of the wildlife fence was approximately 15 cm by 15 cm (height by width), making it permeable to smaller wildlife including rattlesnakes. To prevent rattlesnakes from entering the ROW, ODOT installed a rattlesnake exclusion fence ("snake fence"; 6.35 mm mesh galvanized hardware cloth, 0.9–1.2 m tall, buried < 0.2 m into the ground) along both sides of the highway for 1.6 km spanning the area where rattlesnakes were observed during construction (for maps and photos, see Supplementary Figures 1, 4). The eastern half of the snake fence was attached to the base of the wildlife fence and traversed steep terrain across the ROW-forest boundary, while the western half of the snake fence was built as a free standing fence (detached from the wildlife fence) and traveled closer to the road within the ROW (7–100 m from the road). This western section of the snake fence was rerouted away from the primary wildlife fence to protect a suspected rattlesnake overwintering site in the area.

Five wildlife crossing structures ("ecopassages") were installed along the NVBP. Two of these structures were corrugated steel culverts (52 m long, 1.2 m diameter) designated as small wildlife crossings (SWC) to accommodate smaller wildlife including rattlesnakes. The SWCs featured a grated ceiling at the road median that provided some natural lighting, but conditions within the ecopassage remained poorly lit (for maps and photos, see Supplementary Figures 1, 5). The other three crossings included a gas-line underpass, a large wildlife culvert (e.g. deer), and an all-terrain vehicle underpass. Of the ecopassages along the NVBP, only one of the SWCs interfaced with the snake fence and overlapped the known area of rattlesnake activity. Therefore, 4 of the 5 crossing structures lacked fencing to direct small wildlife such as snakes to the crossing structures.

2.3 Rattlesnake capture

We deployed 32 tin coverboard piles and 12 two-way box traps (Grant et al., 1992; Burgdorf et al., 2005) along the snake fence to live trap and capture rattlesnakes from 2015 through 2017. Traps and coverboards distributed on both the forest and ROW sides of the snake fence. Traps and coverboard piles were spaced 100–150 m apart along the snake fence. Trap placement was partially constrained by fence integrity and terrain. Traps were placed along priority sections of fencing where the fence remained structurally intact, as the fencing was needed to act as a drift fence, diverting snakes to the traps. We avoided placing traps on steep slopes to ensure that traps could be checked safely and would remain flush against the ground. In 2015, traps were distributed along the snake fence on both the westbound and eastbound sides of the highway (6 box traps and 16 coverboard piles on each side of the NVBP). In 2016, all 12 box traps were relocated to the westbound side of the highway due to a lack of trapping success on the eastbound side and higher quality rattlesnake habitat (warmer aspects and den sites) occurring near the snake fencing on the westbound side. Traps were monitored throughout the rattlesnake activity seasons, which began around late April when rattlesnakes emerged (egress) from their winter hibernacula (den) and concluded around early October when rattlesnakes returned (ingress) to their dens. Exact dates of egress and ingress varied from year to year depending on the individual and weather.

2.4 Radio telemetry

We used radio telemetry to detect rattlesnake fence crossings, ecopassages use, mortality and habitat use. Adult and large sub-adult rattlesnakes were implanted with temperature-sensitive Advanced Telemetry Systems® R1680 transmitters (transmitters measured 3.6 g, < 1% body mass for adult rattlesnakes and < 1.5% body mass for subadult rattlesnakes used in our study; Reinert and Cundall, 1982). We used snout-to-vent length (SVL) to determine maturity based on Brown (1991) and Aldridge and Brown (1995) (adult males ≥ 78 cm SVL; adult females ≥ 84 cm SVL). After surgery, snakes were released at the point of capture following a two to seven day recovery period. Snakes were relocated via VHF telemetry (with visual confirmation whenever possible) approximately three times per week (every 48 to 72 hours) throughout the activity season, and GPS locations were recorded using a Garmin® GPS Map 64 (3 m accuracy).

2.5 Additional measures to assess mitigation performance

While radio telemetry was our primary method for monitoring the fate and movement of known individuals, we further evaluated mitigation performance using road mortality surveys and camera monitoring of the ecopassages. We surveyed for road mortality by driving the 13.6 km length of the NVBP in both directions 5 to 7 days/week throughout the rattlesnake activity season (mid-April through mid-October in 2015 and 2016), driving in the right lane at reduced speeds (48–80 kmph depending on traffic conditions) while scouting for rattlesnakes and other reptiles crossing or dead on the road (DOR). After finding a carcass, we documented the species, GPS location, whether it was in the snake fenced area, and when possible, sex and age class. From 2015 through 2016, we used IR Buckeye Game Cameras® placed at both entrances and at the middle of each SWC (three cameras per SWC) to monitor wildlife movement. Cameras were bolted to the ceiling of the culvert, angled downward, and set to detect motion (see the Supplementary Materials for additional information on our analysis of road mortality and wildlife crossing data).

2.6 Evaluating space use

A combination of home range estimation techniques were used to quantify space use relative to the mitigation structures. We generated 100% minimum convex polygons (MCP) for all rattlesnakes each activity season to evaluate whether maximum length of home ranges exceeded the length of snake fencing or occurred in areas that do not overlap snake fencing, which would indicate that the fencing was not sufficient in extent. We also generated 50% kernel density estimators (KDE; Worton, 1989) to calculate the overlap of core activity areas with the ROW to evaluate if important activity centers occurred within the exclusion zone. We generated KDE using the PLUGIN bandwidth operator (Millspaugh et al., 2012). We did not produce KDE home ranges for individuals captured late in the activity season (after early July), as incomplete activity season data would have the potential to misidentify important activity centers. All home ranges were generated in R (version 4.1.2; R Core Development Team, 2022) using the AdehabitatHR package for MCPs (version 0.4.19; Calenge, 2006) and ks package for KDE (version 1.13.5; Duong, 2007).

2.7 Evaluating habitat selection

We contrasted macrohabitat selection and avoidance between gravid and non-gravid timber rattlesnakes using Manly selection ratios under a 3rd order Type II availability design (available habitat pooled across the population; Johnson, 1980; Manly et al., 2002). We considered forest, edge, and right-of-way (ROW) as the major macrohabitat types at the study site (Table 1) and evaluated habitat selection during the period through which females were gravid (June through August). Our use-sample included telemetry relocations from each individual assigned to the respective habitat types. A pooled availability sample was used because home ranges overlapped and lacked natural geographic barriers. The available habitat was defined as follows: 1) 100% MCPs were generated for each snake, 2) a buffer distance equal to the radius of the home range assuming a circular geometry was applied to each MCP (to include available resources immediately outside the observed home range), 3) the buffered MCPs were merged, and 4) clipped at the road because rattlesnakes showed evidence of road avoidance at the NVBP (a correlated random walk analysis on our telemetered snakes is provided in the Road Avoidance section of the Supplementary Materials; timber rattlesnakes have also demonstrated road avoidance in other studies, e.g., Andrews and Gibbons, 2005; Nordberg et al., 2021). Forest, edge, and ROW habitat availability was digitized in ArcMap version 10.4 (ESRI,

TABLE 1 Home range and fence crossing data observed from radio telemetry of timber rattlesnakes at the Nelsonville Bypass.

ID	Year (SVL)	Sex	Relocations (% locations in ROW)	MCP 100% (% overlap with ROW)	KDE 50% (% overlap with ROW)	Fence Crossings	MCP Length
1	2015 (102)	F_G	54 (70.4)	3.6 (30.3)	0.2 (88.9)	5	306
	2016 (103)	F	72 (2.8)	17.1 (1.0)	3.5 (0.0)	0	683
	2017 (103)	F	52 (0.0)	9.9 (0.0)	1.0 (0.0)	0	474
2	2016 (105)	F_G	63 (65.1)	9.7 (11.2)	0.6 (64.4)	6	700
	2017 (105)	F	46 ()0.0	15.0 (0.0)	2.9 (0.0)	0	665
3	2015 (72)	F_S	71 (19.7)	5.7 (7.9)	0.8 (12.5)	4	378
	2016 (77)	F_S	48 (31.2)	8.4 (4.0)	0.6 (5.5)	4	390
4	2015 (93)	M	25 (16.0)	10.4 (4.4)	-	1	749
	2016 (97)	M	75 (17.3)	25.7 (2.3)	2.6 (12.5)	0	937
5	2015 (102)	M	17 (17.6)	6.2 (4.6)	-	NA	753
6	2017 (77)	M_S	52 (9.8)	10.6 (0.1)	1.0 (4.1)	2	678

Sex: M, male; F, non-gravid female; $_S$, sub-adult; $_G$, Gravid. SVL is reported in cm. Relocations = the number of activity season relocations collected, with the percentage of relocations within the ROW reported in parentheses. Home ranges are reported in hectares, with the percentage of area overlapping the "ROW side of the fence" in parentheses. Maximum MCP length is reported in meters, and for comparison, the snake fence spans 1.6 km (1,600 m). We did not generate KDE home ranges for individuals 4 and 5 in 2015 due to insufficient data. Though individual 5 was captured on the ROW side of the fence, fence crossings are not reported because this rattlesnake's home range occurred beyond the snake fence mitigation area.

2016) using aerial imagery (ESRI imagery basemap) and LiDAR canopy data (2.5 m LiDAR collected and made available by the Ohio Geographically Referenced Information Program), where edge habitat was defined as areas within 15 meters of the forest-open canopy ecotone, and ROW habitat included all areas between the road and edge habitat, characterized by an open overstory and other human-made habitat features. Selection ratios were calculated for gravid and non-gravid snakes separately in R using the 'widesII' function in the adehabitatHS package (version 0.3.16; Calenge, 2006), with $\alpha = 0.008$ to correct for multiple comparisons between reproductive classes and among the three habitat types. Selection ratios were interpreted as follows: $w_i > 1$ indicated selection for habitat *i*, $w_i < 1$ indicated avoidance of habitat *i*, and w_i overlapping 1 suggested habitat *i* was used randomly with respect to its availability.

2.8 Thermal habitats and body temperature

We evaluated if thermal conditions influenced habitat selection at the NVBP by comparing habitat types in their availability of gestation and thermally restrictive temperatures. We used operative temperature models (OTMs) to quantify the spatiotemporal availability of ecologically relevant thermal resources across the landscape (Dzialowski, 2005). OTMs mimic an animal's passive heat exchange and can therefore estimate the operative environmental temperature (T_e), often defined as the equilibrium body temperature that an animal would achieve under prevailing environmental conditions in the absence of metabolic heating and evaporative cooling (Dzialowski, 2005). OTMs were constructed from 15.2 cm lengths of copper tubing (3.8 cm diameter, 0.15 cm thickness) and painted to approximate the reflectivity of timber rattlesnakes (e.g., Wills and Beaupre, 2000; Nordberg and Cobb, 2016; Nordberg and Cobb, 2017). Each OTM contained a Thermochron® iButton data loggers (DS1921G ± 0.5°C resolution) that recorded temperature every 20 minutes. OTM design specifications and validation procedures are provided in the Supplementary Materials. In 2016 field season, we distributed OTMs across forest, edge, and ROW macrohabitats (35-39 OTMs per habitat; 110 total) on the ground to quantify operative temperature availability. OTMs were distributed non-randomly at snake activity areas and paired with random walk sites. Activity areas were informed by rattlesnake locations observed through radio telemetry data during the 2015 and 2016 field seasons. Random walk locations were chosen by sampling random distances and bearings from each activity area, and were generated in R using uniform distributions, with distances being sampled from the interquartile range of movement step-lengths observed in telemetry data (10–70 m), and bearings being sampled from 1 to 360°. This model placement was intended to sample ecologically random variation used by, and available to, rattlesnakes at the study site.

Our radio transmitters were temperature sensitive and used to measure snake field active body temperatures (hereafter T_b; see Supplementary Materials). To estimate field preferred gestation T_b (T_g), we used a Lotek SRX 800® (Ottowa, Canada) remote telemetry receiver that monitored T_b in a free-ranging gravid female during the 2016 field season. T_b was recorded every 20 minutes and averaged for mean hourly T_b. We evaluated mean hourly T_b to determine whether average hourly T_b stabilized during photophase (daylight hours), suggesting a field preferred T_b. Due to the rugged terrain and range of our equipment, we were unable to monitor T_b of multiple snakes simultaneously using the remote receiver. To estimate voluntary thermal tolerances (V_{MAX}; Camacho et al., 2018), we considered multiple thresholds: 1) the maximum T_b observed in any of the free ranging telemetered snakes, 2) the maximum T_b observed in a non-gravid snake while not performing an explicitly thermoregulatory behavior (e.g. post-surgery healing, shedding), and 3) values of V_{MAX} reported in the literature (Brattstrom, 1965; Brown et al., 1982). We estimated T_b of all snakes each time they were tracked in the field by measuring the inter-pulse period (Supplementary Materials). In addition, we implanted a male rattlesnake with a Thermochron® iButton data logger (DS1921G ± 0.5°C resolution) that recorded T_b hourly throughout August and September 2016.

After identifying ecologically relevant temperatures (T_g and V_{MAX}), we compared differences among habitats both statistically and graphically. Treating OTMs as the unit of replication, we quantified metrics of daily average thermal conditions for each habitat type including: average T_e, standard deviation (within and among OTMs), maximum T_e, minimum T_e, hours within gestation T_b, hours above average gestation T_b, and hours above V_{MAX}. We summarized these daily indices across habitat types using both the mean and median, and estimated within habitat variation (SD among OTMs). We tested for differences among habitats in the number of hours provided within gestation T_b and exceeding V_{MAX} thresholds using generalized linear mixed effects models, specifying the Poisson distribution (GLMM; Pinheiro and Bates, 2000). Habitat type was modeled as a fixed effect, and OTM identity and ordinal date were modeled as crossed random effects to account variation among models and heteroscedasticity introduced by variation among days. We assessed whether thermal metrics were significantly different among habitats using Tukey *post hoc* tests with Bonferroni corrections, correcting α for the number of among habitat comparisons and thermal metrics tested. Statistical tests were performed in R version 4.1.2 (R Core Team, 2022) using the lme4 package for GLMMs (version 1.1-30; Bates et al., 2015) and multcomp package for multiple comparisons (version 1.4-20; Hothorn et al., 2008). We graphically evaluated mean hourly T_e of each habitat type relative to the mean hourly gestation T_b (monitored gravid female) by plotting means with 95% confidence intervals, and across multiple spatiotemporal scales through heatmaps, area plots, and bar plots, assigning T_e measurements to temperature classes relative to average gestation T_b bounded by standard deviations (SD) and V_{MAX} (Supplementary Material Table 4).

2.9 Gestation habitat resource selection

We compared the spatial availability of gestation habitat within and outside the ROW by modeling a resource selection function (RSF) for gravid females under a use-available design (Manly et al., 2002). Models were fit using generalized linear models (GLM; McCullagh and Nelder, 1989) specifying the binomial distribution and the logit-link function. Use sites were selected as gravid female relocations throughout the summer months (June–August), but because rattlesnakes may remain at the same gestation site for days at a time, relocations were rarefied to include only unique locations. Sites where snakes spent extensive time are likely more important than locations where use was transient, and thus use-locations were weighted relative to the duration of time spent at each location, with the total weight summing to the number of unique locations. The extent of available habitat was defined using the same procedure as for the Manly selection ratios but included only home ranges of gravid snakes (3rd order Type II availability; Johnson, 1980; Manly et al., 2002). Within that extent, we randomly sampled 10,000 availability-locations in R using the SP package (version 1.5-0; Bivand et al., 2013); this sample size was chosen to saturate the available habitat and thus generate representative availability distributions (Northrup et al., 2013). The 10,000 availability points were weighted so that their collective value was equal to the number of use locations (n = 47) to avoid biasing the standard errors of parameter estimates (Barbet-Massin et al., 2012).

Our RSF covariates included: 1) two spatially explicit thermal landscapes; 2) canopy cover (derived from 2.5 m resolution LiDAR collected in 2014 by the Ohio Geographically Referenced Information Program); 3) Continuous Heat Insolation Load Index (CHILI, Theobald et al., 2015; calculated at 2.5 m resolution from a LiDAR derived DEM in Google Earth Engine, Gorelick et al., 2017); and 4) habitat edgeness (i.e., canopy heterogeneity, calculated by taking the standard deviation of a canopy cover raster using focal statistics). We chose these variables based on habitat preferences reported for timber rattlesnakes (Reinert, 1984) and other studies of reptiles in fragmented landscapes where snakes selected forest edges, ostensibly for their warmer temperatures (e.g., Blouin-Demers and Weatherhead, 2002; Waldron et al., 2006; Nordberg et al., 2021; but see also Wittenberg, 2012). Thermal resources are thought to be a fundamental driver of habitat selection in reptiles, and thus T_e surfaces have the potential to model the thermal resource environment directly, whereas heat load, canopy cover, and habitat edgeness are all structural proxies (Huey, 1991; Reinert, 1993; Sears et al., 2011). We modeled thermal landscapes by predicting field collected T_e data using spatial covariates (heat load indices and canopy cover) and weather station data using linear mixed effects models (Fridley, 2009; see Supplementary Materials).

Before multivariable models were developed, we evaluated each covariate in univariable models to identify the optimal scale and functional form (linear versus quadratic) using AICc, retaining the scale and functional form that resulted in the lowest AICc value for each covariate. To avoid multicollinearity, we evaluated correlation structure prior to building multivariable models, and evaluated variance inflation (VIF) for all of our multivariable models. We assessed VIF using the car package (version 3.1-0; Fox and Weisberg, 2019). We modelled thermal landscapes using canopy cover and heat load indices, and thus we avoided building RSF models that included both T_e surfaces in combination with either canopy cover or heat load covariates, as these were highly collinear. The most complex multivariable models in the candidate set were T_e + edgeness and canopy cover + heat load + edgeness. These models were compared with all subsets including univariable forms and a null model. The top model from the candidate set was identified using an information theoretic approach and evaluated based on AICc (Burnham and Anderson, 2002).

After developing the RSF, we evaluated the distribution of gestation resources at the NVBP to determine whether selected habitats were concentrated within the ROW. Across the range of predicted RSF values, we calculated the proportion of modelled habitat of equal or greater value that was contained within the ROW (relative to its availability). To perform these evaluations, the RSF output was rasterized as follows. If the top model was multivariable, we projected it by taking the exponential form of the logistic model and multiplying the model coefficients to their respective covariate raster layers (e.g., Johnson et al., 2006; McDonald, 2013). If the top model was univariable, we would use the raw raster (of the covariate) based on the range of values observed in the use-distribution.

3 Results

3.1 Captures and radio telemetry

Across three field seasons, we monitored box traps for 4,956 traps nights, made 2784 coverboard observations, and spent approximately 3,450 person hours in the field. This effort yielded 501 reptile captures (9 species of snakes, 1 species of turtle, and 1 species of lizard). We captured 18 timber rattlesnakes: four adults (2♂, 2♀), two juveniles (1♂, 1♀), and 12 neonates (5♂, 7♀). We telemetered the four adults and two juveniles and generated 575 relocations over three field seasons (167 relocations of four individuals in 2015, 258 relocations of four individuals in 2016, 150 relocations of three individuals in 2017).

3.2 Mitigation performance

Three of the adult rattlesnakes (2♂, 1♀) were initially captured within the ROW habitats including one male that was captured beyond the linear extent of the snake fencing (i.e., an area of the NVBP that lacked snake fencing). All six telemetered snakes traveled to ROW habitats at least once, and all five of the tracked snakes within the rattlesnake mitigation area crossed the snake fence (22 crossings; Table 1). Snakes apparently crossed through gaps in the snake fence where it was damaged, or traveled over the fence where overgrowth was present. The snake fence was damaged in multiple locations by erosion, corrosion, accumulating debris, tree falls, and overgrowth. Despite the snake fence being a permeable barrier, no telemetered rattlesnakes were killed or injured on the road. We did not observe telemetered rattlesnakes cross or attempt to cross the road directly or by way of the ecopassages. Snake mortality (n = 2; 1♀ subadult, 1 ♂ adult) was caused by predation within the forest based on their carcass locations and wounds. Other species of reptiles were observed dead on the road, including in areas with snake mitigation fencing (Supplementary Materials). While no reptiles were observed crossing the small wildlife crossings, mammals used these structures extensively (see Evaluating the Effectiveness of the Small Wildlife Ecopassages in the Supplementary Materials).

3.3 Space use

We generated 100% MCP home ranges and 50% KDE core activity areas for each individual per year (Table 1), resulting in 11 MCP home ranges from 6 individuals and 9 KDE core areas from 5 individuals. We excluded 2 individuals from KDE analysis in 2015 because they were captured late in the activity season, which would have biased identification of core activity areas. MCP home ranges for rattlesnakes ranged from 3.6–25.7 hectares and maximum home range lengths ranged from 306–937 meters (Table 1; Supplementary Figure 8). All rattlesnakes included both forest and ROW habitats in at least one year, yet all MCP home ranges were apparently bounded by the highway (Supplementary Figure 8). None of the maximum MCP lengths exceeded the length of the snake fence (1.6 km), but one adult female dispersed linearly beyond the extent of snake fencing, and one adult male was captured in the NVBP ROW beyond the snake fence mitigation area (Supplementary Figure 8). Due to incomplete tracking data, we generated KDE home ranges for two individuals in 2015, four individuals in 2016, and three individuals in 2017 (9 home ranges from 5 individuals; Table 1). Six of nine 50% KDE home ranges overlapped the ROW and both gravid rattlesnakes' core home range overlapped the ROW by more than 50 percent (Table 1).

3.4 Habitat selection

Male and non-gravid female rattlesnakes spent most of their time within the forest habitats (68.8–100 percent of relocations on the forest side of the snake fence) while gravid females spent nearly all their time in edge and ROW habitats until giving birth (87.2 to 95.0 percent of relocations). These patterns were reflected in Manly selection ratios, which indicated that gravid females (87 relocations of 2 ♀) selected edge habitat, avoided forest habitat, and use of open canopy ROW habitat was not different from expected use based on availability (overall selection: $\chi^2 = 274.8$, df = 4, $p < 0.001$; Figure 1). Conversely, non-gravid rattlesnakes (273 relocations of 5 individuals; 2 ♂, 3 ♀) avoided ROW habitats, and use of both forest and edge habitats was not significantly different from expected use based on availability (overall selection: $\chi^2 = 91.9$, df = 10, $p < 0.001$; Figure 1).

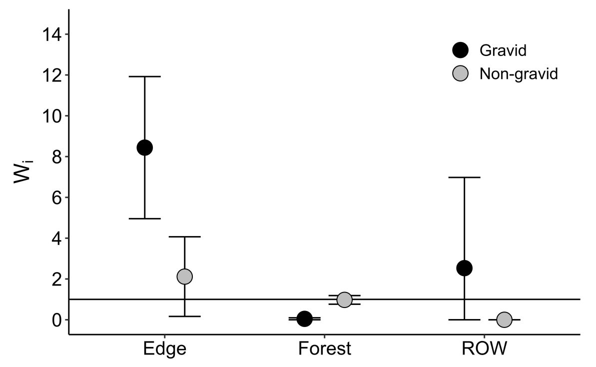

FIGURE 1
Macrohabitat selection for gravid and non-gravid timber rattlesnakes at the Nelsonville Bypass study site. Manly selection ratios were evaluated under a Type II availability design. α and 95% confidence intervals were adjusted for multiple comparisons. W_i are selection ratio coefficients, where $Wi > 1$ suggests positive selection for habitat *i* and $Wi < 1$ suggests avoidance of a habitat *i*. The horizontal line through 1 represents the expected usage of habitat *i* based on its availability (i.e., expected use), and thus confidence intervals overlapping 1 suggest that habitat usage was not statistically different from expected based on its availability.

3.5 Body temperature and thresholds

We recorded 2,754 field active body temperature measurements (hereafter T_b) from 5 rattlesnakes throughout the 2015, 2016, and 2017 activity seasons. We collected 881 hourly T_b measurements from a gravid female using the remote receiver during the warmest period of the summer (26 July to 17 August, 2016) and 1320 hourly T_b measurements from a male rattlesnake with an implanted iButton (2 August to 25 September, 2016). We collected an additional 553 T_b measurements during daylight hours from five radio-tagged rattlesnakes during the three activity seasons, with most observations occurring between May–September, 2015–2017. To compare hourly T_b profiles, we constrained the data to a common range of dates, 2–17 August, 2016. The monitored gravid female maintained a mean hourly T_b range between 26.7–30.4°C (overall mean T_b = 28.7°C, SD = 1.9, n = 626 with 13–35 measurements per hour), which varied considerably less than the range of mean hourly T_e of each habitat which exhibited higher maximum temperatures in the afternoons and lower minimum temperatures in the early mornings (Figure 2A). In contrast, the male rattlesnake implanted with an iButton had a mean hourly T_b range between 22.2–28.6°C (overall mean T_b = 24.9°C, SD = 3.1, n = 384 with 16 measurements per hour), which generally conformed to T_e observed in the forest with a time lag (Figure 2A).

For the gravid rattlesnake, T_b plateaued in the afternoon at about 30°C and mean hourly T_b ranged within 0.5°C from 1500–2100 hours and within 1°C from 1500–2300 hours (Figure 2A). For the period of 1500–2300 hours, mean T_b = 29.7 and SD = 1.8°C, and we thus considered 29.7 ± 1.8°C as the range of field preferred gestation temperatures (hereafter T_g). The maximum recorded T_b, 39.8°C, was a gravid female in 2015 and approaches the critical thermal maximum reported for many reptiles (Brattstrom, 1965). Among non-gravid snakes, the maximum observed T_b was 34.0°C, which approaches the V_{MAX} reported for other crotalids (Brattstrom, 1965). Other timber rattlesnake studies have reported V_{MAX} ranging from 31.5–37.4°C (Brattstrom, 1965; Brown et al., 1982; Wills and Beaupre, 2000). With little consensus among studies, we considered both V_{MAX} values observed in our study for analyses (V_{MAX1} = 34.0°C, V_{MAX2} = 39.8°C), as behavioral thermal tolerances may differ with respect to sex or physiological conditions such as reproductive status.

3.6 Thermal resources

We recorded more than 460,000 T_e readings across 70 summer days in 2016 (n = 110 OTMs, June 21–August 29). ROW and edge habitats provided warmer T_e for longer durations of the day compared with forested habitats (Figures 2, 3). The number of hours T_e occurred within T_g ±1 SD each day was not found to be statistically different among the habitats (Table 2). However, the

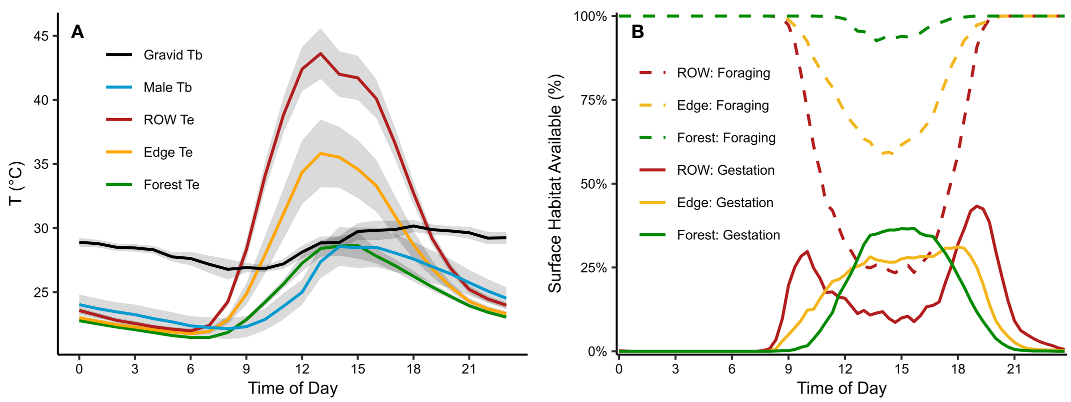

FIGURE 2
Mean operative temperature conditions and surface availability of suitable thermal habitats at the Nelsonville Bypass. **(A)** Mean operative temperatures (T_e) within each macrohabitat along with the mean hourly body temperatures (T_b) from a gravid female and an adult male timber rattlesnake; gray ribbons are 95% confidence intervals. To allow for comparison between curves, T_e and T_b data were summarized across a common range of dates during the warmest period of the summer (August 2, 2016 to August 17, 2016). **(B)** Surface availability of habitat areas at the Nelsonville Bypass based on suitable temperatures for gestation or foraging. Surface availability for gestation was defined as the percent of operative temperature models where T_e was between 27.9 and 31.5°C. Surface availability of foraging habitat was defined as the percent of operative temperature models where $T_e \leq 34.0$°C.

number of hours that T_e reached or exceeded T_g was significantly different among the habitats. T_e reached or exceeded T_g for a mean of 7.8 hours per day in the ROW (median = 8.3), 4.2 hours per day in the edge (median = 4.3), and 1.4 hours per day in the forest (median = 0.3; $P < 0.004$ for all comparisons; Table 2). Fewer than 40 percent of the OTMs within the forest were able to reach T_g for one hour per day on average (median across days by OTM), though approximately 10 percent of forest OTMs were able to meet or exceed T_g for three or more hours per day (maximum = 4.7 hours). In contrast, all ROW OTMs reached or exceeded T_g for a minimum of 4.2 hours per day on average. At any point in the day, median T_e (calculated across all OTMs within a habitat every 20 minutes, Figure 3A) reached or exceeded T_g ±1 SD on 41 of 70 days (58 percent) in the forest, 63 days (90 percent) in the edge, and 69 days (98 percent) in the ROW (Figure 3A). Afternoon T_e in the ROW commonly exceeded voluntary thermal tolerances at the surface throughout the summer, while T_e rarely reached those limits in the forest (Figures 2, 3). T_e exceeded V_{MAX1} for a mean of 5.6 hours per day in the ROW (median = 6.3), 2.5 hours per day in the edge (median = 1.0), and 0.3 hours per day in the forest (median = 0.0; $P < 0.004$ for all comparisons of V_{MAX1} and V_{MAX2}; Table 2). At the hottest hours of the day, only 23 and 39 percent of the ROW OTMs remained below the V_{MAX1} and V_{MAX2} thresholds, respectively, compared to 59 and 72 percent of OTMs in the edge habitat, and 93 and 97 percent of OTMs in the forest (Figures 2, 3). However, gravid rattlesnakes frequently used microhabitat structures within the ROW, especially riprap stone piles and subsurface rock crevices on roadcuts. These microhabitats provided buffered thermal regimes with refugia from extreme temperatures during the day and warmer T_e through the night (Supplementary Figure 10).

3.7 Gestation habitat resource selection

We collected 87 relocations from 2 gravid individuals during the 2015 and 2016 field seasons, which were rarefied to 47 unique use-locations. We generated eight candidate models (Table 3) for the RSF after optimizing scale and functional form of covariates and removing collinear variables (none of the models contained

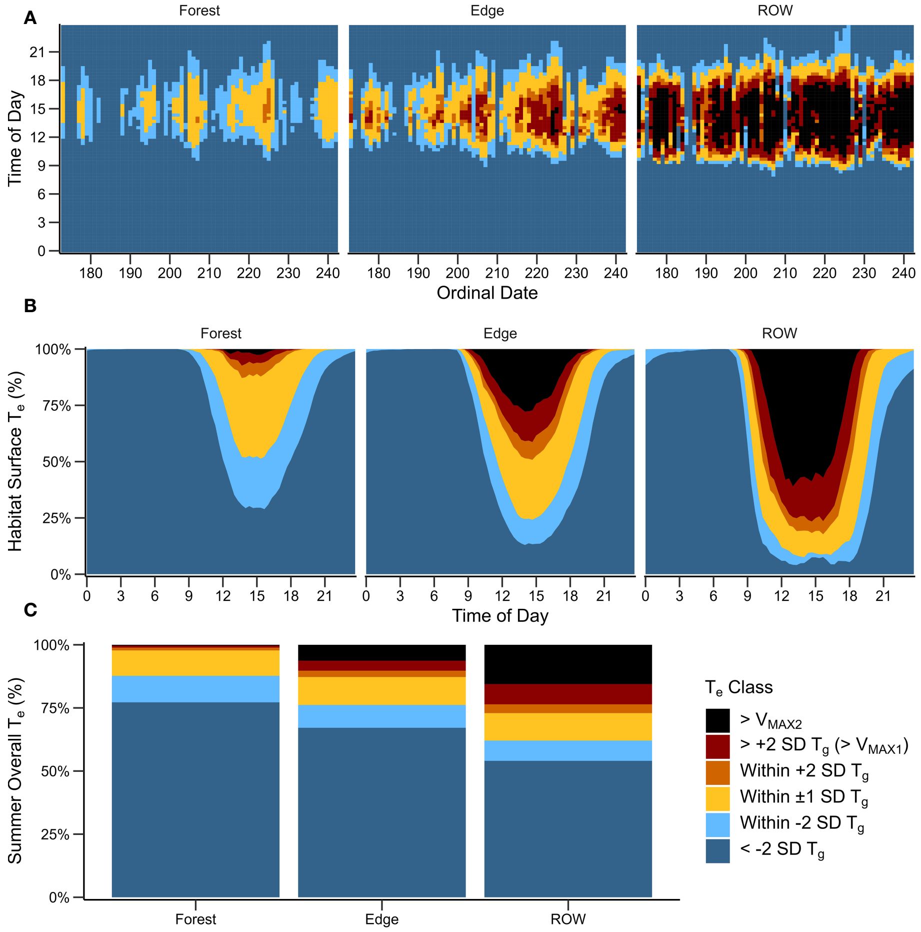

FIGURE 3
Spatiotemporal availability of thermal resources across habitats at the Nelsonville Bypass. T_e classes are in relation to field preferred gestation T_b (29.7°C, SD = ± 1.8°C), and the observed voluntary maximum T_b (V_{MAX2} = 39.8°C). **(A)** Median T_e class available within each habitat for 20-minute intervals from June 21–August 29, 2016. **(B)** Proportion of habitat (OTMs) available within each T_e class across 20-minute intervals for each habitat across the summer. **(C)** Overall proportion of time each habitat experienced a given T_e class across the summer. T_e Classes are described in Supplementary Table 5.

TABLE 2 Summary and comparative statistics for operative temperature data in three macrohabitats at the Nelsonville Bypass.

Daily T_e (°C)	Forest	Edge	ROW
Average	23.3 (23.4)	25.6 (25.1)	28.8 (28.5)
Within Model SD	3.1 (2.8)	5.8 (4.6)	8.4 (8.5)
Among Model SD	2.2 (1.9)	3.5 (2.2)	3.5 (2.6)
Maximum	31.0 (30.0)	40.0 (36.5)	47.1 (48.0)
Minimum	19.6 (20.0)	19.7 (20.5)	20.3 (21.0)
Daily Duration (Hours)			
T_e within T_g ±1 SD	*2.3 (1.7)	*2.5 (2.0)	*2.4 (2.0)
$T_e \geq T_g$	*1.4 (0.3)	**4.2 (4.3)	***7.8 (8.3)
$T_e > V_{MAX1}$	*0.3 (0.0)	**2.5 (1.0)	***5.6 (6.3)
$T_e > V_{MAX2}$	*0.1 (0.0)	**1.5 (0.0)	***3.7 (4.0)

With the exception of among model SD, each daily metric was calculated for each OTM individually and for each day, and then summarized by calculating the mean and median (in parentheses) across OTMs. Among Model SD was calculated by taking the standard deviation of daily average mean or median (in parentheses) T_e across all the OTMs in each habitat. For daily metrics reported in hours, T_e within T_g ± 1 SD = $27.9 \leq T_e \leq 31.5$; $T_e \geq T_g = T_e \geq 29.7$; $T_e > V_{MAX1} = T_e > 34.0$; $T_e > V_{MAX2} = T_e > 39.8$. Post hoc evaluations adjusted α to 0.004 to correct for multiple comparisons (0.05 ÷ 12, as 12 = 4 tests × 3 groups). The number of asterisks (*) indicates which habitats were found to be statistically similar for a given metric, such that habitats with differing numbers of asterisks were found to be significantly different at α = 0.004.

variables with VIF > 1.1). The top model identified by AIC_C was a univariable model that contained T_{eAVG}, and identified that gravid snakes preferred locations on the landscape with higher T_{eAVG} (odds ratio = 3.56, ß = 1.27, SE = 0.23, $P < 0.001$, McFadden's Pseudo $R^2 = 0.57$). Models were not averaged due to issues that would have arisen with multicollinearity (Cade, 2015), but also because alternative models were not competitive with the top model ($\Delta AIC_C \geq 3.57$). Gravid female use-availability plots suggested positive selection for locations where $T_{eAVG} > 25$°C (Figure 4). Approximately 91 percent of the gravid female use locations occurred at locations where $T_{eAVG} \geq 25$°C despite these areas representing only 23 percent of the available habitat area (Figure 4). Within the available habitat extent (63.6 hectares), 94 percent of the ROW area (9.0 of 9.5 hectares) exhibited $T_{eAVG} \geq 25$°C, compared to only 9 percent of the forest (5.1 of 54.1 hectares), suggesting that thermal habitats preferred by gravid rattlesnakes were concentrated within the ROW.

4 Discussion

We found that exclusion fencing at the NVBP failed to exclude rattlesnakes from the ROW, with rattlesnakes repeatedly crossing in and out of the ROW, likely through damaged sections of the exclusion fence. Similar to other road mitigation studies, these failures resulted from design specifications including improper materials, fence placement and extent, and lack of maintenance (Baxter-Gilbert et al., 2015; Huijser et al., 2016). For most of its length, the fencing at the NVBP was built at the edge of the ROW (as far as 100 meters away from the road in some locations), ostensibly to identify the ODOT-National Forest property boundary, where it sustained significant damage. Gaps were created beneath the fence by washouts on steep slopes, blowouts where soils and plant matter accumulated on eroding road cuts and forested hillsides, and corrosion in areas with acidic soils (Supplementary Figure 6). Rattlesnakes could have also passed

TABLE 3 Resource selection function (RSF) models evaluated for gravid female timber rattlesnakes at the Nelsonville Bypass.

Model	K	AIC_C	ΔAIC_C	Weight	LL	Pseudo R
T_{eAVG}	2	39.8	0.00	0.72	−17.91	0.57
Can	2	43.3	3.57	0.12	−19.69	0.53
Can + HL + HL^2	4	43.5	3.69	0.11	−17.75	0.57
Can + HL + HL^2 + Edge	5	46.6	6.85	0.02	−18.33	0.56
T_{eAVG} + Edge	3	47.00	7.18	0.02	−20.50	0.51
HL + HL^2	3	69.13	29.32	0.00	−31.57	0.24
Edge	2	76.29	36.48	0.00	−36.15	0.13
Null	1	85.18	45.36	0.00	−41.59	0.00

T_{eAVG} = average daily temperature landscape, which was modeled by predicting OTM data from weather station data combined with spatially explicit data from the study site. Can = canopy cover averaged using a 25 m radius focal window; HL = heat load averaged using a 75 m radius focal window, Edge = habitat edgeness, which was generated by taking the standard deviation across a binary canopy cover layer using a 5 × 5 cell focal window, and then smoothed by averaging across a 10 m radius focal window. Original rasters for all layers were sampled at 2.5 m resolution obtained through LiDAR. McFadden's pseudo R^2 is reported, which is a metric of log likelihood improvement over the null model.

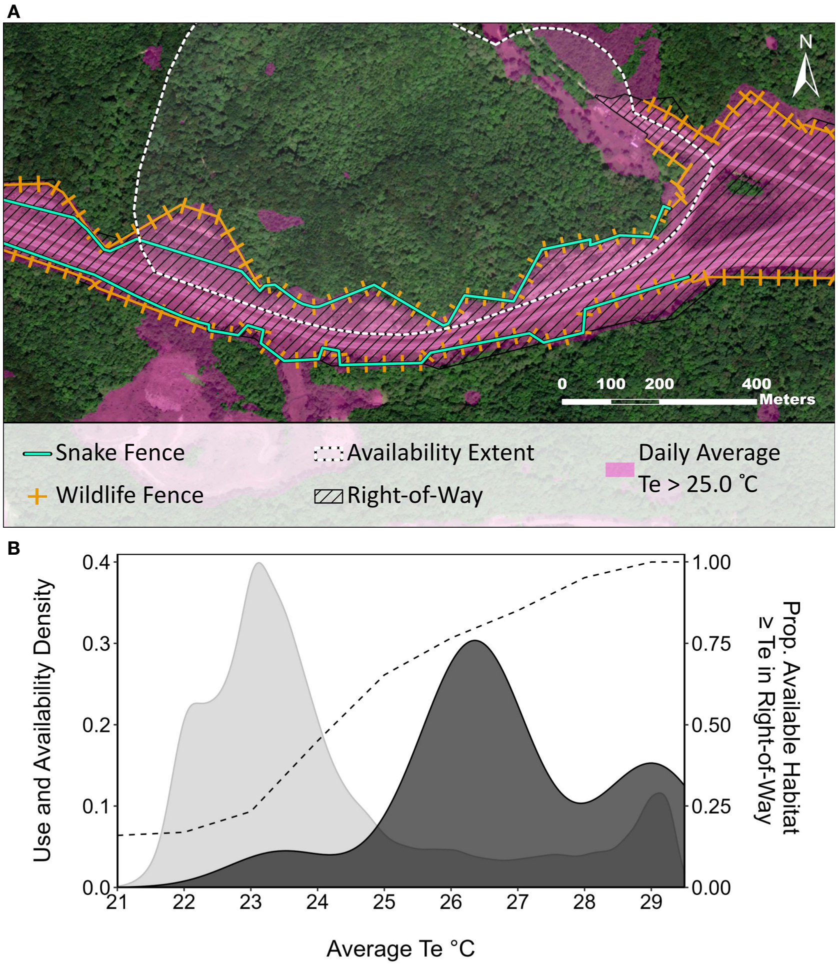

FIGURE 4
Thermal resources availability relative to the road corridor. **(A)** Map of the Nelsonville Bypass in the area available to gravid rattlesnakes, showing the location of exclusion fencing relative to modelled thermal habitats selected by gravid females (Average T_e > 25°C). **(B)** Density plots of the use sample (dark gray) and availability distributions (light gray) for values on the Average T_e thermal landscape, which was identified as the most important covariate in gravid rattlesnake resource selection functions. The dashed line shows the proportion of the available habitat area that meets or exceeds a given T_e value and occurs within the Nelsonville Bypass ROW.

over the fence by climbing overgrowth and tree falls that crushed the fence (Supplementary Figure 6). Nonetheless, nine rattlesnakes were captured in boxtraps or under coverboards along the snake fence indicating that the fence did divert movement in locations where it remained structurally intact. Unfortunately, annual maintenance did not prevent the fence from falling into disrepair, and damages along the fence went ignored in numerous places.

We did not observe evidence of rattlesnakes using the crossing structures to maintain population connectivity across the highway. Ultimately, we could not determine whether rattlesnakes or other reptiles did not use the crossing structures due to flawed mitigation design, unfavorable conditions within the crossings, the small population size (low probability of rattlesnakes encountering the crossing), or inadequate camera sensors. The damaged fencing and its poor interfacing with the crossing structures inhibited our ability to meaningfully evaluate whether the crossing structures could maintain rattlesnake population connectivity. Only one of five crossing structures at the NVBP, a SWC, was interfaced with the snake fence. In addition, the SWC that was interfaced with fence was properly interfaced with the fencing on only the northside of the NVBP, as the fencing ran above and behind the culvert entrance on the southside of the NVBP (Supplementary Figure 5). The snake fencing on the northside of the bypass, where rattlesnakes were tracked, was intact for approximately 100 meters on either side of culvert entrance. At least three of the rattlesnakes we tracked came within 50 meters of the crossing structure, and one individual was initially captured along the fence within five meters of the crossing structure. However, none of the rattlesnakes we tracked showed repeated usage of habitats within 100 meters of the crossing. Considering the gaps in the exclusion fence, the small population size, and the limited rattlesnake activity near the crossing structures, the NVBP rattlesnakes likely had few, if any, encounters with the crossing structures.

Other studies have shown difficulty detecting reptiles and amphibians using Passive IR cameras, and it is possible that we too failed to detect successful crossings that could have occurred (Hobbs and Brehme, 2017; Pomezanski and Bennett, 2018). However, we find it unlikely that we missed timber rattlesnake crossings given that we detected multiple species of reptile much smaller in size at the entrances of these crossings. We find it more likely that rattlesnakes did not use the structures due to the fencing and interfacing design flaws identified above. In addition, other rattlesnake studies have observed reluctance to cross through structures similar to those built at the NVBP (Colley et al., 2017; Laidig and Golden, 2004). The conditions throughout the SWCs were poorly lit and score low by metrics of openness (i.e., low width: length ratio; Yanes et al., 1995). While the NVBP crossing structures were used extensively by mammals, rattlesnakes and other reptiles were not observed crossing the structures. Rattlesnakes and other reptiles may have been deterred from crossing by chemosensory signs of predatory mammals that frequented the structures. Raccoons (*Procyon lotor*) and Virginian opossums (*Didelphis virginiana*) were frequently observed in the crossings and are known snake predators (Supplementary Materials).

The use of ROW and edge habitats, overlap of core home ranges with the ROW (Table 1), and neutral to positive selection for those human-made habitats by gravid females (Figure 1) all suggested that the NVBP ROW provided important habitat for the rattlesnakes. Using operative temperature modelling, we observed warmer thermal regimes available in the edge and ROW habitats (Figures 2, 3) and found that landscape temperatures selected by gravid females were concentrated within the habitats created by road construction (Figure 4). Daytime T_e in the ROW often exceeded T_g, but the warmer T_e profiles of the ROW extended the temporal availability of preferred thermal habitat for gravid females relative to the forest. While mean hourly forest T_e approached T_g ±1 SD during midday hours, edge and ROW habitats would often reach and exceed T_g for four to eight hours of the day (Figure 2; Table 2) with the ROW showing peaks in T_g availability in the morning and evening hours (Figure 2B). Within the ROW, gestation sites used by gravid females were exposed to continuous full sun during the day, but were located at deep rock crevices (holes and fissures emerging from the roadcuts) or stone piles (drainage control riprap). These microhabitats provided thermal refugia that allowed snakes to escape extreme temperatures during the afternoon and maintain elevated T_b through the night as T_e dropped above-ground (Supplementary Figure 10). These microhabitat features within the ROW provided a broad thermal gradient that facilitated precise thermoregulation throughout gestation (Figure 2; Supplementary Figure 10). The combination of open canopy, south facing slopes, diverse microhabitats, and rocky retreats within the ROW apparently created preferred gestation sites where rattlesnakes could maintain elevated body temperatures throughout the day and night.

With afternoon temperatures routinely exceeding V_{MAX} in the ROW (Figures 2, 3), much of this area would have been unsuitable for sit-and-wait ambush foraging which was reflected in the avoidance of ROW habitats by non-gravid snakes (Figure 1). Female rattlesnakes that made use of the ROW through gestation did not return to the ROW after giving birth in the same activity season, and did not return to ROW in their post-partum activity season except during ecdysis. Non-gravid snakes crossed the exclusion fence infrequently, but when they did, they spent most of their time in edge habitats at the fringe of the ROW while exhibiting behaviors linked to thermoregulation such as shedding or healing from injuries (e.g., transmitter replacement). The two adult males we tracked both traveled to the ROW or edge habitat during ecdysis until shedding. Thus, both gravid and non-gravid rattlesnakes were apparently crossing the snake fence to access warmer habitats available at the forest edge and within the ROW. The landscape matrix surrounding the NVBP is primarily forested, and we speculate that basking and gestation sites are more limited than foraging habitat. For this reason, the benefits of the novel thermal habitats created by the ROW may outweigh the costs of lost foraging habitat. However, because the mitigation structures do not exclude rattlesnakes from the roadway nor maintain connectivity, eventual road mortality and genetic drift may detrimentally affect the rattlesnake population over time (Rudolph et al., 1999; Clark et al., 2010; Bushar et al., 2015).

Despite detecting a significant number of Eastern Box Turtle (*Terrapene carolina*) road mortalities at the NVBP (Supplementary Materials), we did not observe rattlesnake road mortality via either radio telemetry or road mortality surveys. Our vehicle speeds were not ideal for rigorous monitoring of road mortality (minimum speeds were required by ODOT to avoid creating hazardous traffic conditions), which may have limited our ability to detect small reptile carcasses. However, carcasses of turtles and large snakes were easily visible on the road because the surface of the highway was still relatively new, providing visual contrast (Supplementary Figure 7). Correlated random walk simulations indicated that rattlesnake movements were consistent with road avoidance (Supplementary Materials), which echoes numerous studies where timber rattlesnakes have demonstrated an aversion to crossing roads based on experimental trials (Andrews and Gibbons, 2005) and telemetry data collected in road fragmented landscapes (Nordberg et al., 2021; Tipton et al., 2023). Road avoidance in other species has been shown to scale with the road size and traffic intensity (Brehme et al., 2013), and the NVBP is a four-lane divided highway, with high traffic volumes (exceeding 17,000 vehicles per day) traveling at high speeds (112 km/hr). If rattlesnakes did attempt to cross the NVBP, the probability of road mortality would be high because timber rattlesnakes cross roads slowly and often pause for oncoming traffic (Andrews and Gibbons, 2005). The small rattlesnake population at the NVBP and the long generation times of timber rattlesnakes suggest that additive road mortality could cause rapid population decline or extirpation.

Our study site was selected based on the location of the mitigated highway, which was co-located with a small and endangered population of rattlesnakes. Working with this small population severely limited our sample size of rattlesnakes. While small sample sizes require caution, our study does not claim any novel discoveries regarding timber rattlesnake spatial ecology, habitat selection, or thermal biology. Our observations are consistent with the ecology of the species established by decades of past field research (Reinert, 1984; Reinert et al., 1984; Reinert and Zappalorti, 1998; Waldron et al., 2006; Gardner-Santana and Beaupre, 2009; Nordberg et al., 2021; Tipton et al., 2023), including our observations of field active body temperatures and roadside habitat use (Reinert and Zappalorti, 1998; Gardner-Santana and Beaupre, 2009). Yet, our study is valuable to the road ecology conversation because it is among few that have examined the intersection of spatial, thermal, and road ecology (e.g., Peaden et al., 2017). Specifically, our spatially explicit characterization of thermal habitat resources allowed us to examine how the distribution of those resources likely influenced movement and habitat selection with respect to roadside habitats and mitigation fencing. The structural design failings of the snake exclusion fence, use of ROW habitats by rattlesnakes, the quantification and comparison of thermal habitat resources, and the physical properties that likely attracted rattlesnakes to the ROW are all observations that may be applicable to timber rattlesnake population management and road mitigation planning in general.

4.1 Roads and thermal habitat resources

At the NVBP, road construction introduced resource heterogeneity to the landscape that ostensibly motivated fence crossings by attracting rattlesnakes to the forest edge and ROW. Our results are echoed in many other studies that have observed use of, or preference for, roadsides, edge habitats, and other ROWs (Langen et al., 2015). Use of, or preference for, human-made ROWs has been reported for many species of reptiles across broad geographies including snakes and lizards in North America (*Elaphe obsoleta*, Blouin-Demers and Weatherhead, 2002), Europe (*Zamenis longissimus*, Kovar et al., 2014; *Chameleo chameleon*, Hódar et al., 2000), South America (*Ameiva ameiva*, Vitt et al., 1998; Sartorius et al., 1999), and Australia (*Egernia major*, Klingenböck et al., 2000; *Bassiana duperreyi*, Shine et al., 2002). Another commonality among these studies was that reptiles were apparently drawn to these modified habitats to access thermal resources, often at the expense of increased mortality risk, and sometimes with population level consequences. For example, it has been reported widely that open roadside habitats often attract female turtles for nesting (Haxton, 2000), which can result in ecological trap formation and female biased mortality (Aresco, 2005; Steen et al., 2006).

At the NVBP, the ROW provided a large corridor of open canopy and rocky microhabitats within a closed canopy forest matrix that was used by rattlesnakes and other reptiles. Structurally, these landscape changes were analogous to habitat improvement techniques used for reptiles in thermally limited environments. For example, at local scales, forest canopy thinning was an effective restoration technique to improve habitat quality and reptile species diversity at sites in Australia and North America (Webb et al., 2005; Pike et al., 2011; Hromada et al., 2018). Similarly, many thermophilic reptiles use microhabitats characterized by rock outcroppings for their physical and thermal properties (Reinert, 1984; Huey et al., 1989; Croak et al., 2008). These species can be affected by the removal or shading of rocky habitats (Shine et al., 1998; Pike et al., 2011), and benefit from their restoration and sunning (Croak et al., 2010; Pike et al., 2011). Multiple studies have reported negative effects of forest succession and canopy closure on temperate reptile populations and communities, which resulted in the declines and extirpations of some snake and lizard populations (Hall, 1994; Ballinger and Watts, 1995; Jäggi and Baur, 1999; Fitch, 2006).

Simulations and experiments predict that ectotherms can thermoregulate more effectively and efficiently in landscapes with small and dispersed patches of thermal resources as opposed to landscapes with fewer but larger patches of thermal resource (Sears et al., 2016). Yet, our study and others have shown that large disturbances can create thermal habitats that are otherwise uncommon or absent in small canopy gaps (Vitt et al., 1998; Sartorius et al., 1999). Specifically, large canopy openings and broad east-west clearings allow for extended hours of direct solar radiation for basking. Analogous human-made ROWs come in a variety of forms aside from road corridors, such as electric, gas, and other utility corridors. These linear features can have similar ecological properties given that they are also maintained in an open canopy or early successional state. As observed in our study and others, the thermal habitats created by these ROWs are sometimes sought by reptiles (e.g., Vitt et al., 1998; Sartorius et al., 1999), which may be of consequence to population vital rates given the range of behaviors and physiological processes governed by thermal biology (Angilleta, 2009).

We acknowledge that most roadsides exist as bare or mowed grass features and lack the diverse microhabitat features we observed at the NVBP. Roadsides often exist as degraded habitats that favor weedy species, introduce edge-effects harmful to forest interior species, and can create ecological traps (Chalfoun et al., 2002; McKinney, 2006; Langen et al., 2015). However, there are instances where ROWs contain suitable microhabitats such as road cuts, stone piles, and downed logs, which may have value to reptile populations if there is a deficit of basking habitat resources in the surrounding landscape. We hypothesize that ROWs are more likely to attract reptiles where they create a stark resource gradient with the surrounding landscape, for example, in wide ROWs created in forested landscapes, where reptiles (or other ectotherms) have limited basking sites. Conversely, narrow ROWs and landscapes where the creation of a ROW does not create a stark resource gradient, as in arid, treeless landscapes and urban environments, would be less likely to attract reptiles. These novel resource gradients may be most consequential in landscapes where past timber practices or fire suppression have resulted in more homogenous forest conditions with closed canopy structure. Again, we consider these cases to be exceptions to the rule, and that the value of such ROWs to wildlife is contingent on the presence of suitable habitats contained within the corridor and the absence of ecological traps.

4.2 Management recommendations and conclusions

Our observations of flawed mitigation design and rattlesnake behavior in roadside habitats resulted in two site-specific recommendations at the NVBP. First, building an exclusion fence 5 to 10 meters from the road, along the length of the bypass, and interfaced with crossing structures (e.g., Langton and Clevenger, 2021) would better protect the NVBP timber rattlesnake population, eliminate the most common sources of structural damage, and facilitate routine maintenance of the fence. We reiterate that the continued effectiveness of any mitigation fencing requires regularly scheduled maintenance and upkeep that should be planned for in design and budgeting. Second, because the thermally exposed habitat features selected by gravid rattlesnakes will continue to undergo succession, the population would also benefit from the restoration and maintenance of basking sites (canopy gaps with stone piles, rock crevices, or large hollowed logs as refugia) within the surrounding forest on the habitat-side of the fence.

We do not advocate the construction of roads through landscapes as a form of habitat enhancement because wildlife populations are often adversely affected by roads (see Introduction). However, when roads and other ROWs are to be built through wildlife habitat, it is worth considering the landscape context and affected resource environments (e.g., thermal, forage, or shelter resources), how wildlife may respond to novel resources, and whether mortality or other adverse effects are likely to increase during use of, or movement to and from, those resources (i.e., ecological trap formation). These same considerations are also worth visiting when designing wildlife fencing (Jakes et al., 2018). Habitat heterogeneity, particularly where stark resource gradients are formed between the ROW and the surrounding landscape, has the potential to introduce strong resource selection pressures. In cases where such resource gradients are present, fence placement may be consequential to species attracted to those resources, and particularly when ROW resources are associated with the reproductive success of a given species. When roads introduce open canopy habitats and rocky features to forested landscapes, we recommend evaluating the potential value of these habitat features to local reptile populations, whether those features can be maintained on the habitat-side of exclusion fencing, and whether basking habitats could be restored away from the road to lessen the selection pressure for roadsides habitats and reduce the probability of ecological trap formation. Understanding whether these resource selection pressures are likely to form may help design more effective road mitigation for reptile populations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted in accordance with the local legislation and institutional requirements. The animal study was approved by Ohio University Institutional Animal Care and Use Committee (approval issued to WR under protocols 14-L-018 and 13-L-023). The study was also conducted under the Scientific Collection Permit #18-84 issued by the Ohio Department of Natural Resources.

Author contributions

WR and GS conceived the study and wrote the grants. GS and WR developed the study design. GS conducted the field work and analyzed the data. GS wrote the manuscript with input from WR. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. All authors contributed to the article and approved the submitted version.

Funding

The study was funded by the Ohio Department of Transportation under ODOT Agreement 26656 CFDA 20.205 and ODOT Agreement 27695.

Acknowledgments

We would like to thank Matt Perlik, Mike Austin and the Ohio Department of Transportation, Lynda Andrews and the Wayne National Forest, and Ohio Division of Wildlife for their support of this project. We thank Viorel Popescu for substantial analytical feedback, as well as Shawn Kuchta and Joseph Johnson for their comments on earlier versions of this manuscript. We thank Seth Cones, Isabel Fisk Baruque, Christine Hanson, Kelly Johnson, Adam Kabrick, Tyler Stewart, and Aspen Wilson for their dedicated assistance in the field. Finally, we would like to thank the reviewers and editor for their feedback which greatly improved the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1059461/full#supplementary-material

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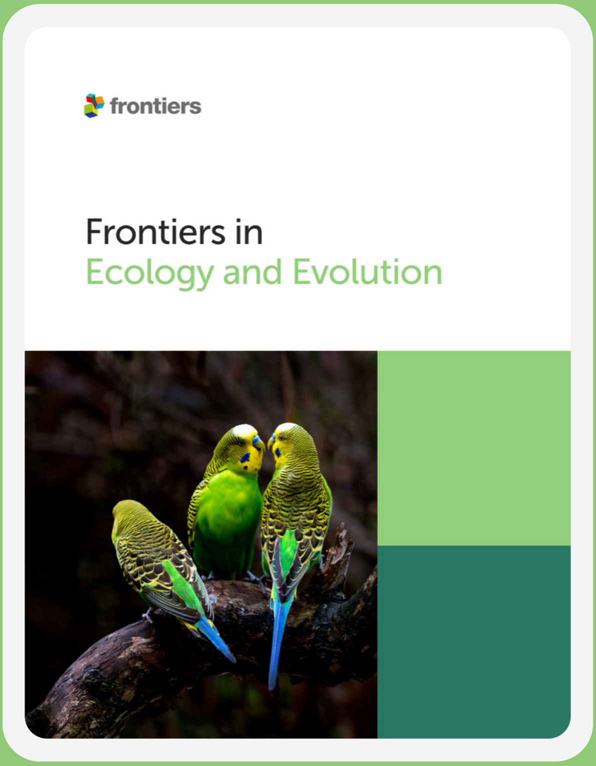

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