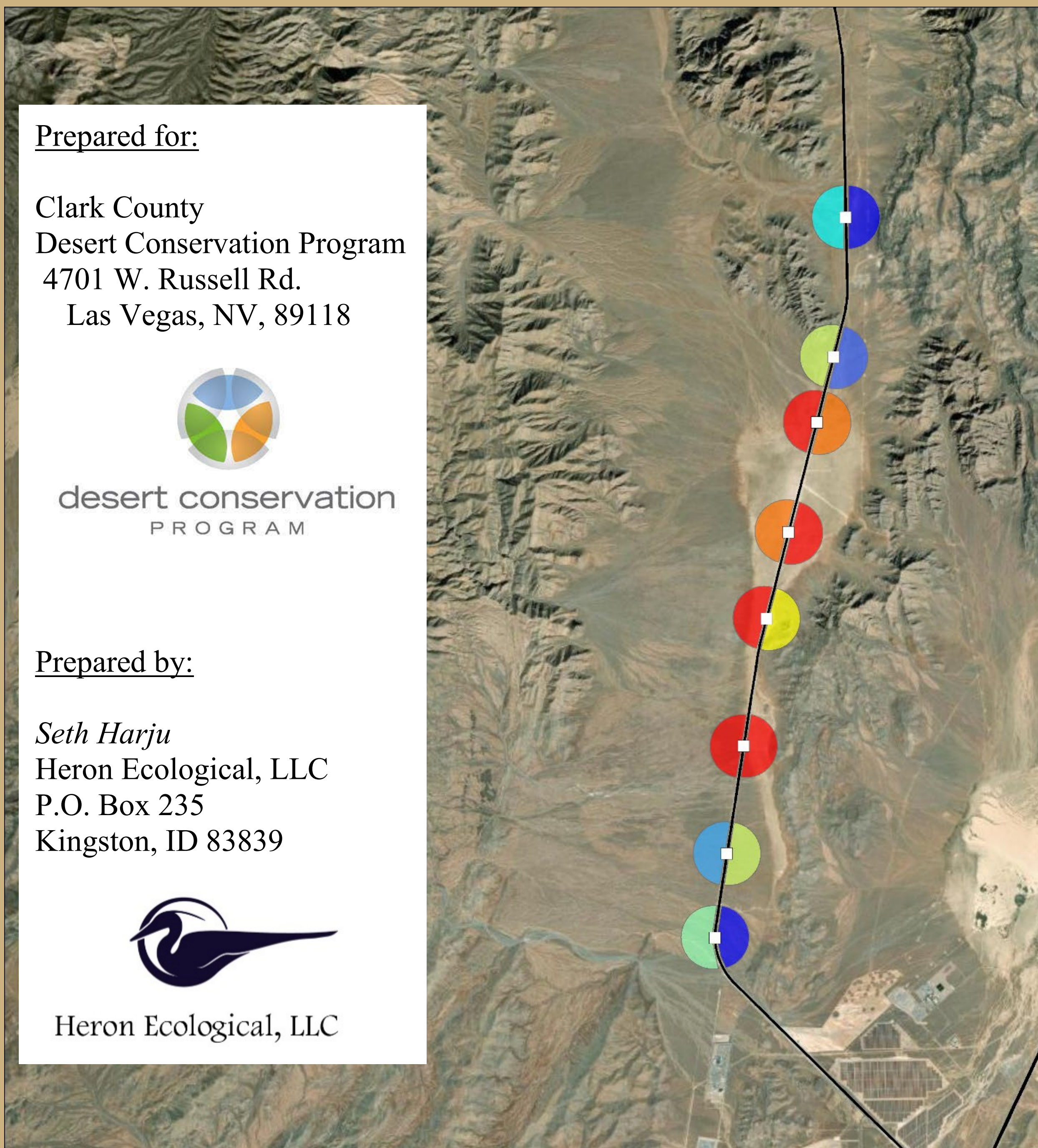
# MOJAVE DESERT TORTOISE CONNECTIVITY DATA ANALYSIS

## Data Deliverable D04

## Project 2015-HERON-1580J



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## INTRODUCTION

Roads impact a wide variety of wildlife species both directly and indirectly. Direct impacts can include significant direct mortality and, to a lesser degree, direct habitat loss. Indirect impacts are farther reaching and often less quantified, including buffer zones of avoidance by wildlife (e.g., indirect loss of habitat) and facilitated spread of invasive species and predators. For Mojave desert tortoises (*Gopherus agassizii*), the impact of roads via direct mortality is well known. Current management practices call for exclusionary fencing along highways to prevent tortoise ingress on to the highway, which has reduced direct mortality (Sadoti et al. 2017). While beneficial to tortoise populations via reductions in road mortality, large-scale exclusionary fencing has the unintended consequence of creating population fragmentation, whereby long-term gene flow, genetic connectivity, and population connectivity (e.g., dispersal) are curtailed. To remedy this curtailment, culverts under roadways are often placed with the hope that they facilitate tortoise movement under fenced highways, thus restoring connectivity (Boarman et al. 1998). Culvert use by tortoises has been documented, but such documentation is rare and the frequency of tortoise use of culverts for crossing is poorly understood.

The indirect impacts of roadways on tortoises are less understood. Previous work has found that Mojave desert tortoise populations have lower abundance and are generally comprised of smaller juveniles near major roadways (Nafus et al. 2013). Roads (and associated barrier fences) also affect movement, with tortoises moving less when activity centers are closer to minor roads and barrier fences (Sadoti et al. 2017). Nonetheless, tortoises are known to use culverts to cross highways (Boarman et al. 1997). What is missing is a mechanistic link between fenced roadways, connectivity culverts, local densities of tortoises, and tortoise movement behaviors.

To better understand the direct use of culverts and the indirect effects of a fenced highway and connectivity culverts on a local tortoise population, the Clark County Desert Conservation Program initiated a multifaceted study along U.S. Highways 93 and 95 in northern Clark County, Nevada, USA. The study involved mark-recapture of free-ranging tortoises, installation of camera traps on existing culverts, and capture and attachment of Global Positioning System (GPS) units on free-ranging tortoises to monitor movement behaviors and culvert crossing rates of GPS-tagged tortoises. The goal of the study was to estimate tortoise densities on either side of culverts, to record tortoise movements using GPS data, and to document culvert use via mark-recapture, photos, and GPS data.

## MATERIALS AND METHODS

### Field data collection

Mark-recapture surveys were conducted by Ecocentric, LLC (Valentine 2021) March 24, 2021 and May 19, 2021. Survey plots were designed as two plots per culvert, each being a semicircle with 800 m radius from the culvert on either side of the culvert (i.e., either side of the highway). There were eight plot pairs placed on U.S. Highway 93 (Hwy 93) and ten plot pairs established along U.S. Highway 95 (Hwy 95). Culvert types were different between the two highways, with Hwy 93 having cylinder culverts (24-36 in diameter) and Hwy 95 having large box culverts. Survey transects within each plot (i.e., 800 m radius semicircle) were 10 m apart. Each plot was surveyed by a team of two to five technicians, within a maximum three-day span, aiming for 100% coverage of the plot. Each plot was surveyed three times within the spring survey window. See Valentine (2021) for additional details and results from field surveys.

Camera traps were placed at culvert entrances and were operational from April through September of 2019 and 2020. Cameras recorded pictures when tortoises approached the culvert. If a tortoise approached or entered the culvert but did not exit the other side the event was classified as a 'resting' event. If the tortoise was captured on photo entering a culvert on one camera and exiting the culvert on the other camera, the event was classified as a 'crossing'. Camera crossing data were prepared and provided by S. Cambrin with the Clark County Desert Conservation Program.

The Clark County Desert Conservation Program also contracted field crews to survey, capture, GPS tag, and release Mojave desert tortoises along U.S. Highway 95 during spring and summer of 2021. Tortoise searches and captures were restricted to 800 m radius semicircles centered on the north and south sides of eight existing culverts along the highway (i.e., they targeted tortoises with a reasonable chance of crossing the nearest culvert). GPS units were set to record location coordinates every ~30 minutes. Tortoises were relocated in the field via VHF approximately every 7-10 days to determine mortality status.

## Statistical analysis

### *Density*

I used closed population maximum likelihood capture-recapture models to estimate Mojave desert tortoise abundance. I first tested a set of simple candidate models evaluating whether probability ($p$) of detecting an individual tortoise was constant, varied among plot surveys, or varied between cardinal location of the plot relative to the highway (e.g., north side, south, east, or west). For most models I assumed that probability of recapture ($c$) was equal to detection probability (i.e., $p = c$), based on the survey methodology and desert tortoise behavior (e.g., tortoises were not 'surveyor happy' or 'surveyor shy' after their initial capture), but also tested two models where tortoises may have had a behavioral response to initial capture. Finally, I tested whether the abundance of uncaptured tortoises ($f0$) was equal across all plots or was different as a function of the cardinal location of the plot. Models for $p$ and $c$ were intended only to obtain the best fit to the data; models for $f0$ tested the primary hypothesis of interest, whether plots on one side of the highway had consistently higher or lower abundances than the other side. Significant cardinal differences would suggest a barrier effect of roads, preventing population admixture. Total abundance (derived $\widehat{N}$) was derived by combining the number of captured tortoises within the plot with the estimated number of uncaptured tortoises $f0$.

After testing for the importance of a plot's cardinal location relative to the highway, I tested models for $p$ and $c$ that estimated $f0$ separate for each plot. This generated plot-level $\widehat{N}$ abundance estimates which were used for further analyses of barrier effects of the two highways. All abundance estimation assumed that the plot's population of tortoises was closed across the three surveys in spring 2021 (e.g., no permanent emigration/immigration or births/deaths). Model selection was via Akaike's Information Criterion corrected for small sample size (AICc). All abundance estimation was carried out in Program MARK (v 8.1) via the RMark package in Program R (v 4.0.5).

I conducted analyses using the plot-level abundance estimates from the capture-recapture data to further test for barrier effects of the roads. First, I tested whether abundance was consistently higher on either side of each highway using two-sample t-tests. If we assume equal habitat conditions along all surveyed sections of each highway, then significant differences on either side would indicate a lack of population admixture. However, habitat is often not of uniform quality over large distances. Therefore, second, I used paired t-tests to assess whether within each plot pair, there were significant differences in abundance between either side of the highway. I did this separately for each highway, and thus for each culvert type (i.e., cylinder versus large box).

After calculating density estimates, I tested whether the number of observed camera crossings was related to mean tortoise density on both sides of the culvert, the maximum density on either side of the culvert, or the absolute difference in the density of tortoises between both sides of the culvert using negative binomial regression. Tests of mean and maximum density evaluated whether crossing rates were a function of tortoise abundance (i.e., more tortoises equals more crossings). The test of absolute difference in density evaluated whether there was a gradient effect, whereby tortoises crossed the highway in response to a gradient in density (i.e., higher disparity in tortoise density equals more crossings). Models were compared using AICc. Density and crossing analyses were conducted using the 'MASS' package in Program R (v 4.0.5).

*Movement*

First, I quantified culvert crossing rates of the GPS-tagged desert tortoises as the number of crossings per tortoise-days monitored. This provided some basic comparable information on how frequently tortoises residing near culverts actually crossed through culverts.

Second, I used Hidden Markov Models (HMM) to assign tortoise GPS locations to either a single behavioral movement state or to one of two mutually exclusive behavioral movement states: resting and moving. I used Akaike's Information Criterion (AIC) to compare the null single-state model to the two-state model. The choice of two states was based on the availability of two data streams from the GPS units (i.e., derived steplengths and turning angles), the fact that more than two movement states are difficult to statistically discern from only two data streams (McClintock et al. 2017), and based on examination of the sequential GPS location data from the tagged tortoises (Figure 1). The resting state encompassed any localized behaviors, including resting, feeding, basking, burrowing, aestivating (e.g., during summer), etc. The moving state encompassed all moving behaviors, such as foraging, dispersal, mate-seeking, etc. After selecting the two-state model I ran 25 two-state model analyses using a range of randomly chosen starting values to ensure appropriate statistical convergence (Michelot et al. 2016).

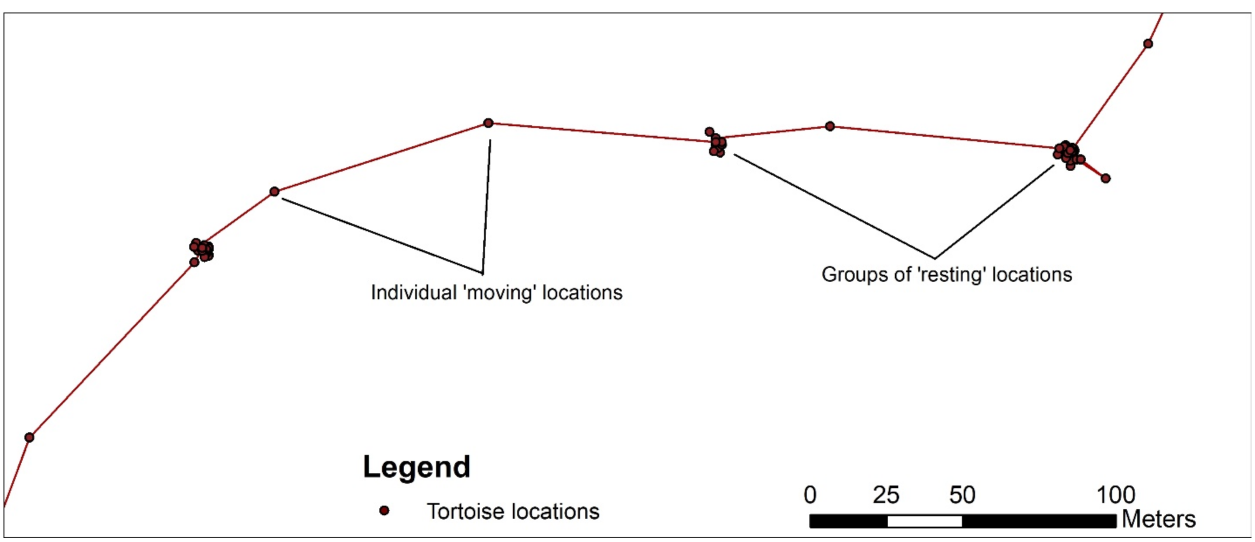


**Figure 1.** Sample of locations from Tortoise ID CC0677, highlighting two apparent movement states, with individual locations a member of either a resting or moving state.

For the two-state HMM analyses I evaluated four baseline biological movement models, testing whether movement states were best explained via a null model, whether transitioning between movement states was a factor of the time of day, whether movements within either movement state was affected by time of day, and whether both transitions between and movement within movement states were affected by time of day. I then used the best model from this initial set of biological movement models to evaluate whether observed movement behaviors were further explained by how close each tortoises' location was to the highway, including whether proximity to the highway influenced the transition rate between or movement behavior within each movement state and whether this effect was nonlinear. Competing models were evaluated using AIC and relative weights of evidence in favor of the models that were tested (Burnham and Anderson 2002). HMMs were built using package 'momentuHMM' in Program R (v4.0.5).

## RESULTS

*Density*

There were 272 captures of 183 individual tortoises across all surveyed plots. Of the 16 plots along Hwy 93, nine plots had ≥ 1 tortoise captured and five plots had 0 tortoises captured. Along Hwy 95, 14 plots had ≥ 1 tortoise captured and six plots had 0 tortoises captured.

Closed population mark-recapture model selection found overwhelming support for models that had different abundances of tortoises depending on whether a plot was on the north, south, east, or west side of the highway (Table 1). Amongst the top models, the model with detection probability varying across survey occasions and with recapture probability being equal to detection probability was best supported (Akaike weight = 0.84). This suggests that for some cardinal locations of plots, roads may have been acting as barriers to population admixture.

**Table 1.** Model selection results for models comparing closed population abundance models with detection probability (p), recapture probability (c), and unmarked abundance of Mojave desert tortoises (f0). AICc is an information criterion to select the most parsimonious model for the data from among the candidate set. DeltaAICc ≥ 4 are considered to be models not competing with the top model.

| Model No. | Model | No. Parm. | AICc | DeltaAICc | weight |
|---|---|---|---|---|---|
| 8 | p(~time)c()f0(~Plot_dir) | 7 | -431.414 | 0 | 0.84 |
| 4 | p(~1)c()f0(~Plot_dir) | 5 | -427.383 | 4.030838 | 0.11 |
| 2 | p(~1)c(~1)f0(~Plot_dir) | 6 | -425.505 | 5.908106 | 0.04 |
| 6 | p(~Plot_dir)c()f0(~Plot_dir) | 8 | -421.698 | 9.716021 | 0.01 |
| 5 | p(~Plot_dir)c()f0(~1) | 5 | -420.212 | 11.20164 | 0.00 |
| 7 | p(~time)c()f0(~1) | 4 | -414.068 | 17.34533 | 0.00 |
| 1 | p(~1)c(~1)f0(~1) | 3 | -410.605 | 20.80906 | 0.00 |
| 3 | p(~1)c()f0(~1) | 2 | -409.499 | 21.91455 | 0.00 |

When calculating plot-specific abundance estimates, the model with detection probability varying among sampling occasions and recapture probability being constant was best supported (Akaike weight = 0.70), with weak support for a model where detection probability was constant and there was a behavioral effect of initial capture, whereby tortoises were less likely to be captured again following their initial capture (ΔAICc = 2.40; Akaike weight = 0.21; Table 2). For our purposes, I ignored the second-best model as our goal was simply plot-level derived $\widehat{N}$ abundance estimates.

**Table 2.** Model selection results for models comparing closed population abundance models with detection probability (p), recapture probability (c), and unmarked abundance of Mojave desert tortoises (f0) separate for each survey plot. AICc is an information criterion to select the most parsimonious model for the data from among the candidate set.

| Model No. | Model | No. Parm. | AICc | DeltaAICc | weight |
|---|---|---|---|---|---|
| 4 | p(~time)c()f0(~PlotID) | 28 | 210.2558 | 0 | 0.70 |
| 1 | p(~1)c(~1)f0(~PlotID) | 27 | 212.6597 | 2.403964 | 0.21 |
| 2 | p(~1)c()f0(~PlotID) | 26 | 214.4598 | 4.204093 | 0.09 |
| 3 | p(~PlotID)c()f0(~PlotID) | 50 | 236.5571 | 26.30138 | 0.00 |

Derived $\widehat{N}$ abundance estimates for each plot along Hwy 95 indicated that plots south of the highway had significantly higher estimated abundances of tortoises than plots north of the highway ($t = -5.01$, $df = 8.49$, $p < 0.001$), with the exception of the second most easterly plot, which had zero tortoises both north and south of the highway (Figure 2). Southern plots had a mean estimate of 7.11 tortoises, northern plots had a mean estimate of 1.20 tortoises, with southern plots on average having 5.80 (95% CI 3.18 – 8.42) more tortoises than their paired northern plots. Plots along Hwy 93 did not show consistent differences on either side of the highway ($t = 0.71$, $df = 8.82$, $p = 0.499$), and in general for plots with tortoises present had higher tortoise abundances than plots along Hwy 95 (Figure 3).

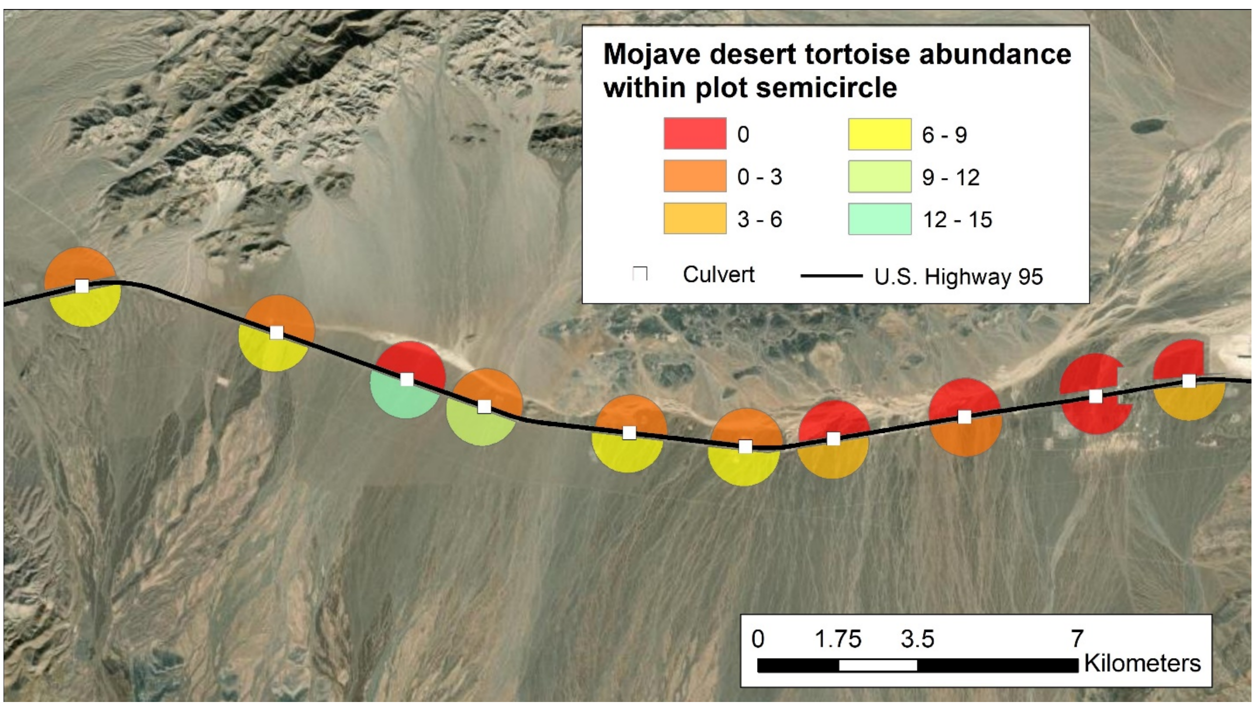


**Figure 2.** Derived $\widehat{N}$ Mojave desert tortoise abundance estimates for survey plots along U.S. Highway 95, southern Nevada.

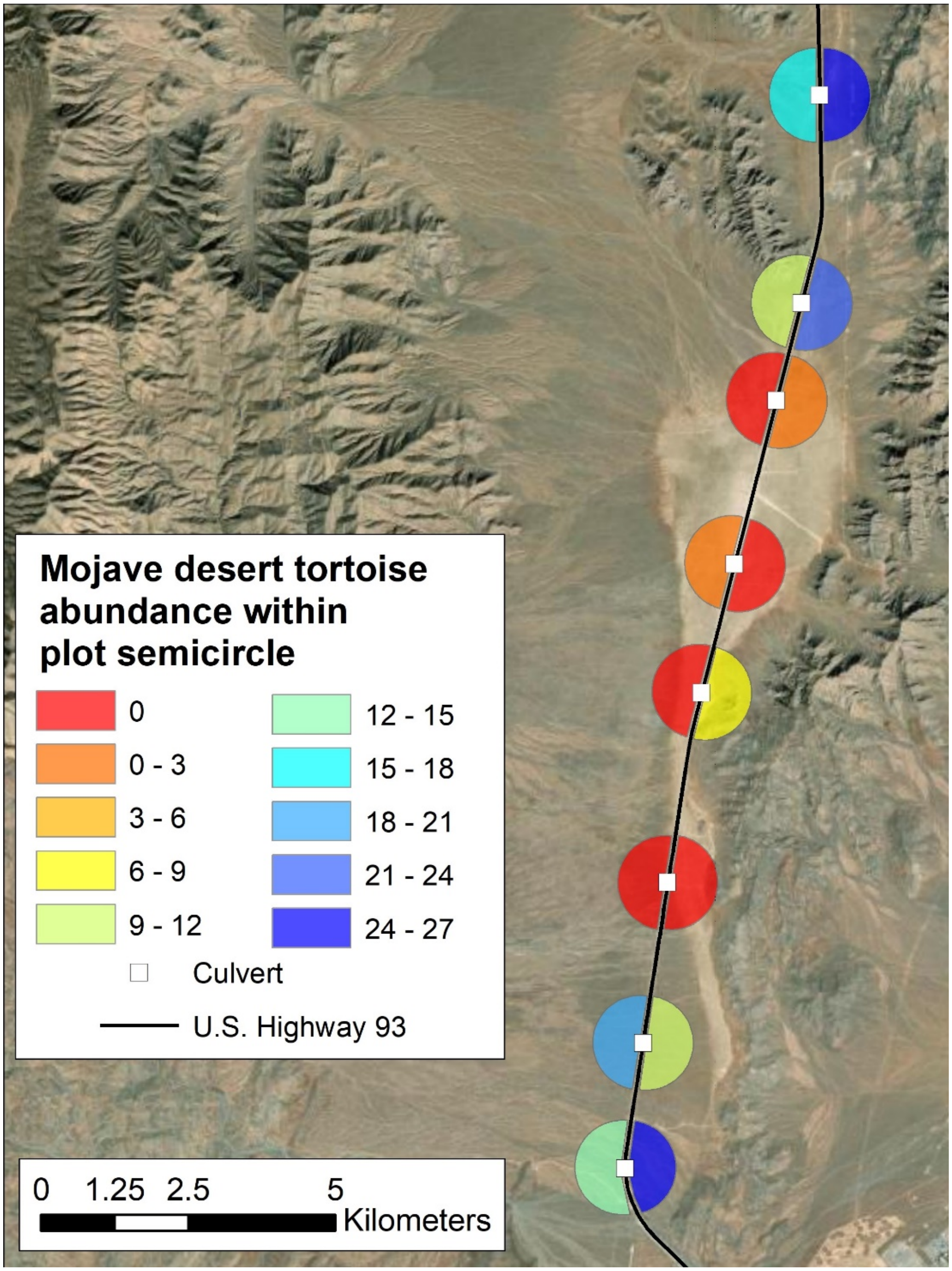


**Figure 3.** Derived $\widehat{N}$ Mojave desert tortoise abundance estimates for survey plots along U.S. Highway 93, southern Nevada.

There were a total of 22 highway crossings observed via camera traps and 49 events of culvert usage for 'resting' without a crossing. Tortoises crossed both corrugated box (72 in) and corrugated metal (36 in) culverts, but most frequently crossed corrugated metal culverts and in general did not cross all culvert types available (Figure 4). Oppositely, tortoises rested in all types of culverts, including all sizes of corrugated box and corrugated metal culverts (Figure 5). The total number of highway crossings was positively associated with the absolute difference in tortoise density on either side of the highway ($\beta = 0.567$, se = 0.216, p-value = 0.009), but not on mean total tortoise density ($\Delta$AICc from null model $\leq$ 1.29; Table 3). This means that more tortoises on average did not equal more crossings. Rather, higher crossing frequency was associated with increasing disparity in tortoise densities on either side of the highway (Figure 6).

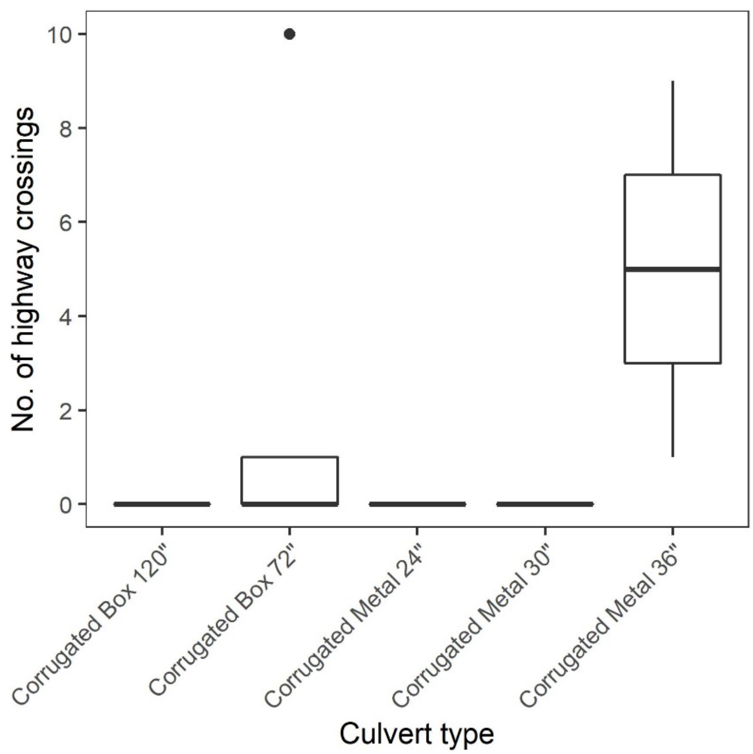


**Figure 4.** Number of highway crossings separated by culvert type. Horizontal bars are the median number of visits, boxes are interquartile ranges, lines are maximum and minimum observed values (if ≤ 1.5 * interquartile range), and dots are outlier observations.

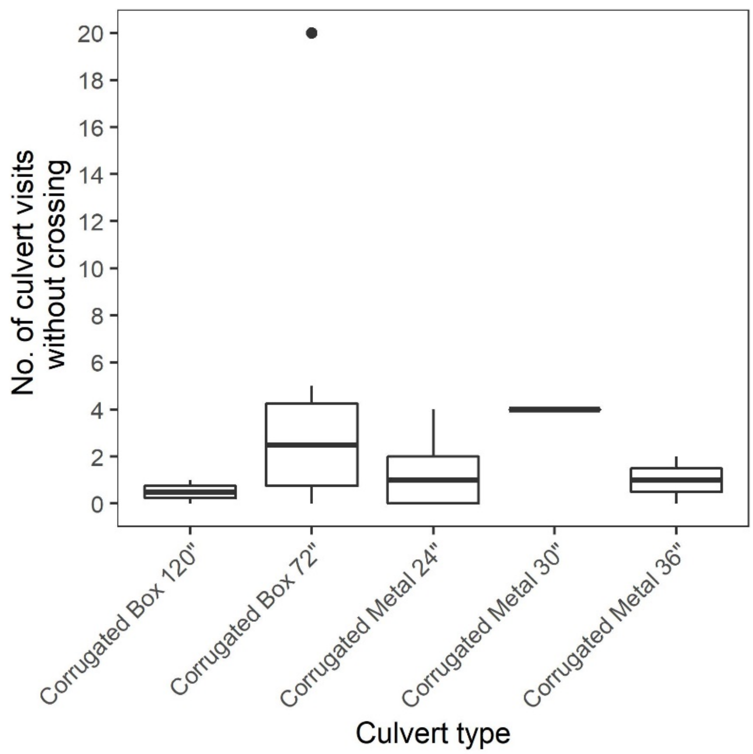


**Figure 5.** Number of culvert 'resting' visits that did not result in crossing the highway. Horizontal bars are the median number of visits, boxes are interquartile ranges, lines are maximum and minimum observed values (if ≤ 1.5 * interquartile range), and dots are outlier observations.

**Table 3.** Model selection results for factors explaining total number of highway crossings.

| **Model** | **AICc** | **ΔAICc** |
|---|---:|---:|
| Difference in density | 43.92 | 0 |
| Null | 48.2 | 4.28 |
| Maximum density | 48.48 | 4.56 |
| Mean density | 49.49 | 5.57 |

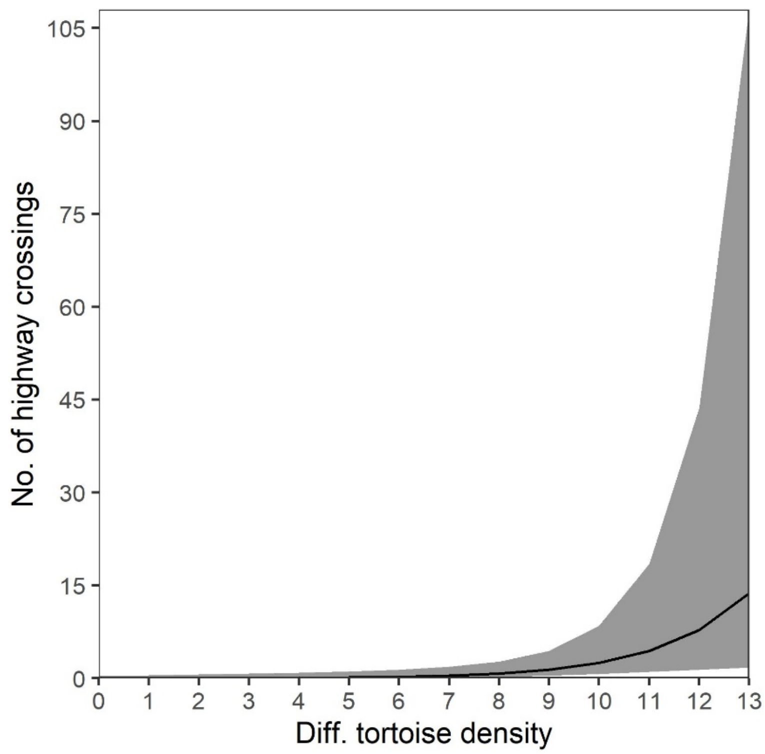


**Figure 6.** Predicted number of highway crossings as a function of absolute difference in tortoise density (number of individuals per plot) on either side of the highway.

*Movement*

Fifteen adult resident tortoises (nine males and six females) were captured and outfitted with GPS units. Fourteen tortoises were captured between 4/3/21 and 5/14/21; the fifteenth tortoise was captured on 8/19/21. There was a total of 1,298 tortoise-days with recorded GPS locations. Of these, there were only two crossing events: a single male crossed from the north side of culvert 95-7 at mile marker 131.5 to south of the highway on April 14, 2021 and returned through the same culvert back to the north side of the highway on June 30$^{th}$, 2021. Tortoise locations were generally between 200 – 1,000 m from the highway (Figure 7).

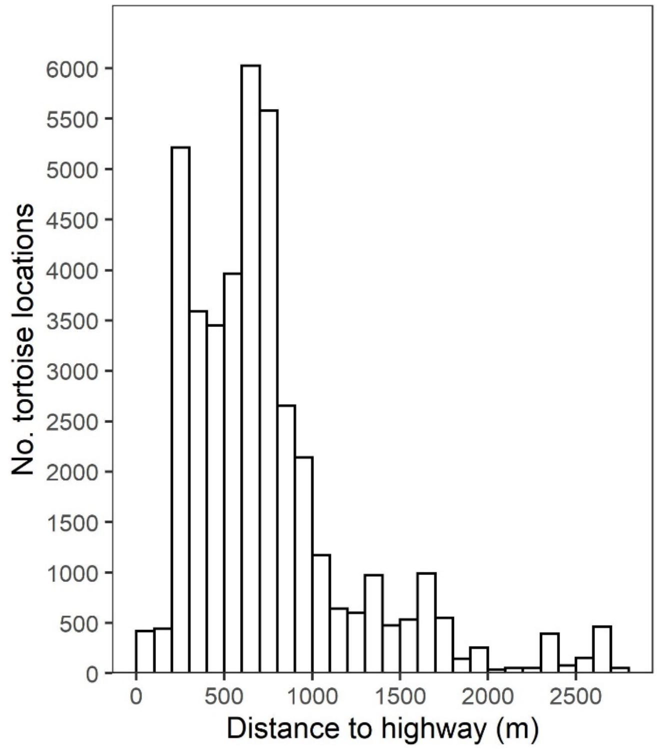


**Figure 7.** Histogram of distances from each Mojave desert tortoise GPS location to U.S. Highway 95 in northwestern Clark County, Nevada, April 3rd though October 9th, 2021.

Initial assessment of a baseline biological movement model found overwhelming support for a model with two underlying behavioral states versus a single state ($\Delta$AIC = 33,413.8). Most distances between consecutive GPS locations (i.e., steplengths) were short, although tortoises had longer steplengths when in the moving state than when in the resting state (Figure 8). Mean steplength in a moving phase was 43.7 m (95% CI 41.8 - 45.6 m), which was an order of magnitude longer than mean steplength during the resting phase (4.5 m, 4.4 - 4.6 m). Turning angles also varied between movement states, with tortoises in the moving state having uniformly distributed turning angles and those in the resting state strongly recursive (Figure 9). Further assessment found strong support for a model where movement behavior within both resting and moving movement states, and transition rates between either state, were non-linear functions of the time of day ($\Delta$AIC $\geq$ 220.9; Table 4). Tortoises exhibited strong non-linearity in both steplengths and variability in steplengths with both steplength distances and variability peaking between 10:00 and 16:00 hours in the afternoon for both resting and movement states (Figure 10).

**Table 4.** Model selection results for models comparing baseline biological movement models for 15 resident adult Mojave desert tortoises. AIC is an information criterion to select the most parsimonious model for the data from among the candidate set. DeltaAIC ≥ 4 are considered to be models not competing with the top model. The variable 'weight' is the relative weight of evidence in favor of a given model as being the best model from within the candidate set.

| Model | AIC | DeltaAIC | weight |
|---|---|---|---|
| Within-state (time of day) + Transition (time of day) | 375693.9 | 0 | 1.00 |
| Within-state (time of day) + Transition (null) | 375914.8 | 220.9 | 0.00 |
| Within-state (null) + Transition(time of day) | 376481.0 | 787.1 | 0.00 |
| Within-state (null) + Transition (null) | 377051.5 | 1357.6 | 0.00 |

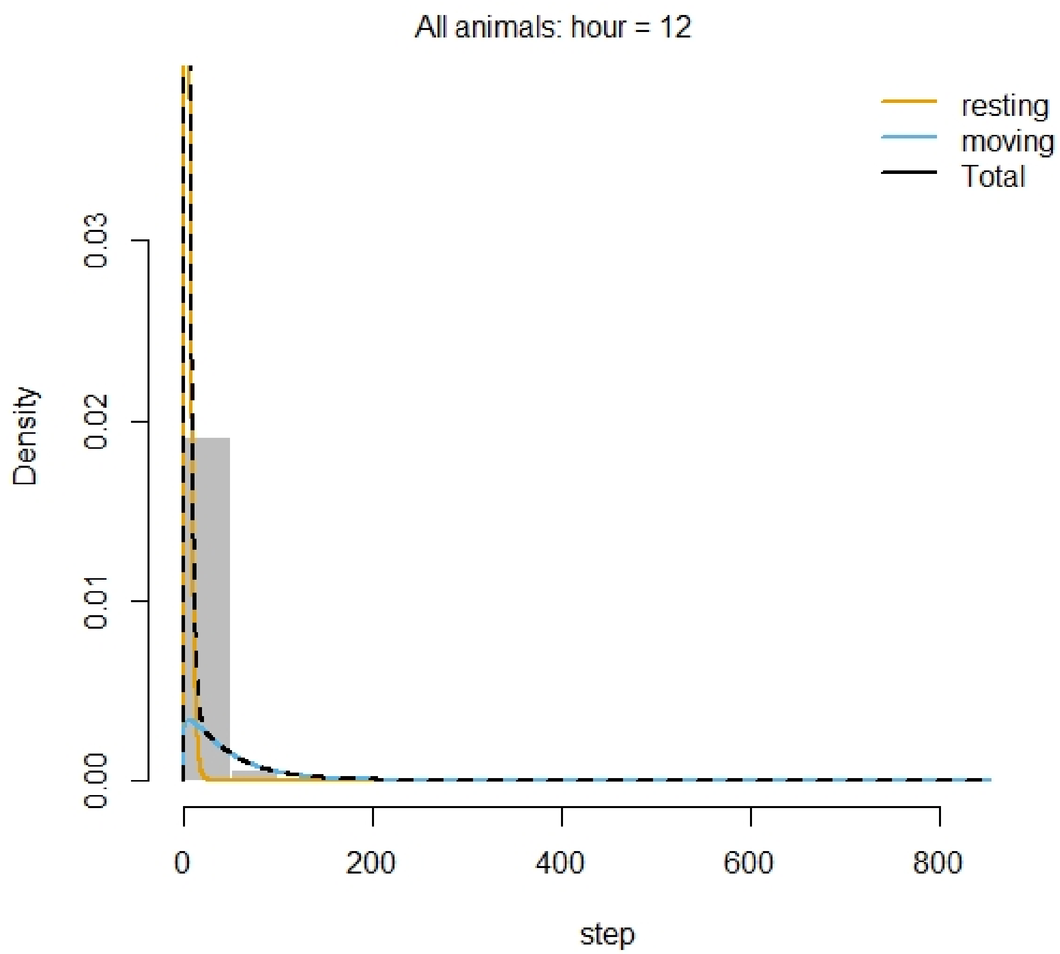


**Figure 8.** Distributions of steplengths between consecutive locations (~30 min duration) of Mojave desert tortoises along U.S. Highway 95 in northwestern Clark County, Nevada. Displayed distributions are steplengths at noon (hour = 12).

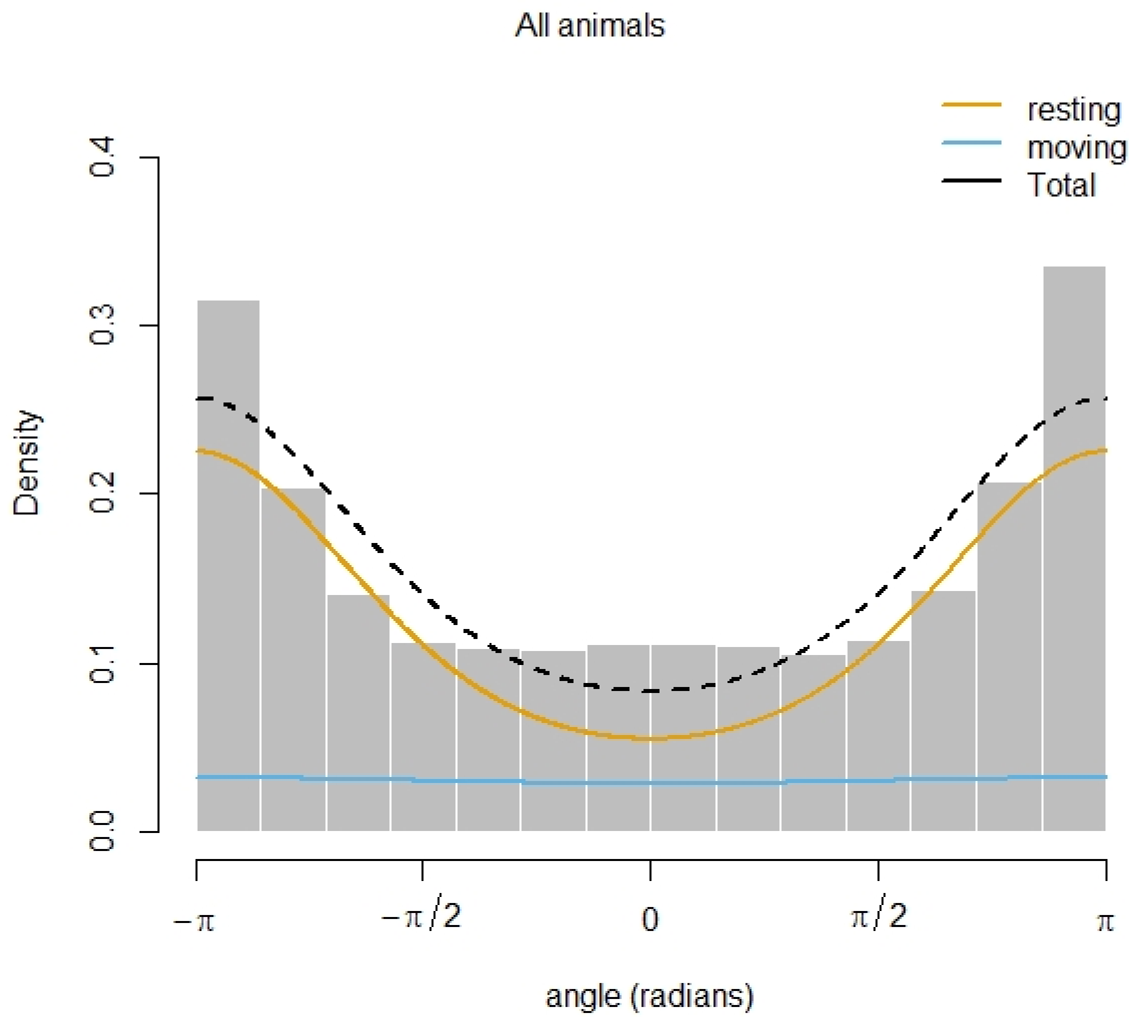


**Figure 9.** Distributions of observed turning angles for Mojave desert tortoises in resting and movement behavioral states. Angles of 0 radians indicate straightline consecutive steps and angles of |π| indicate 180° turns (i.e., recursive steps).

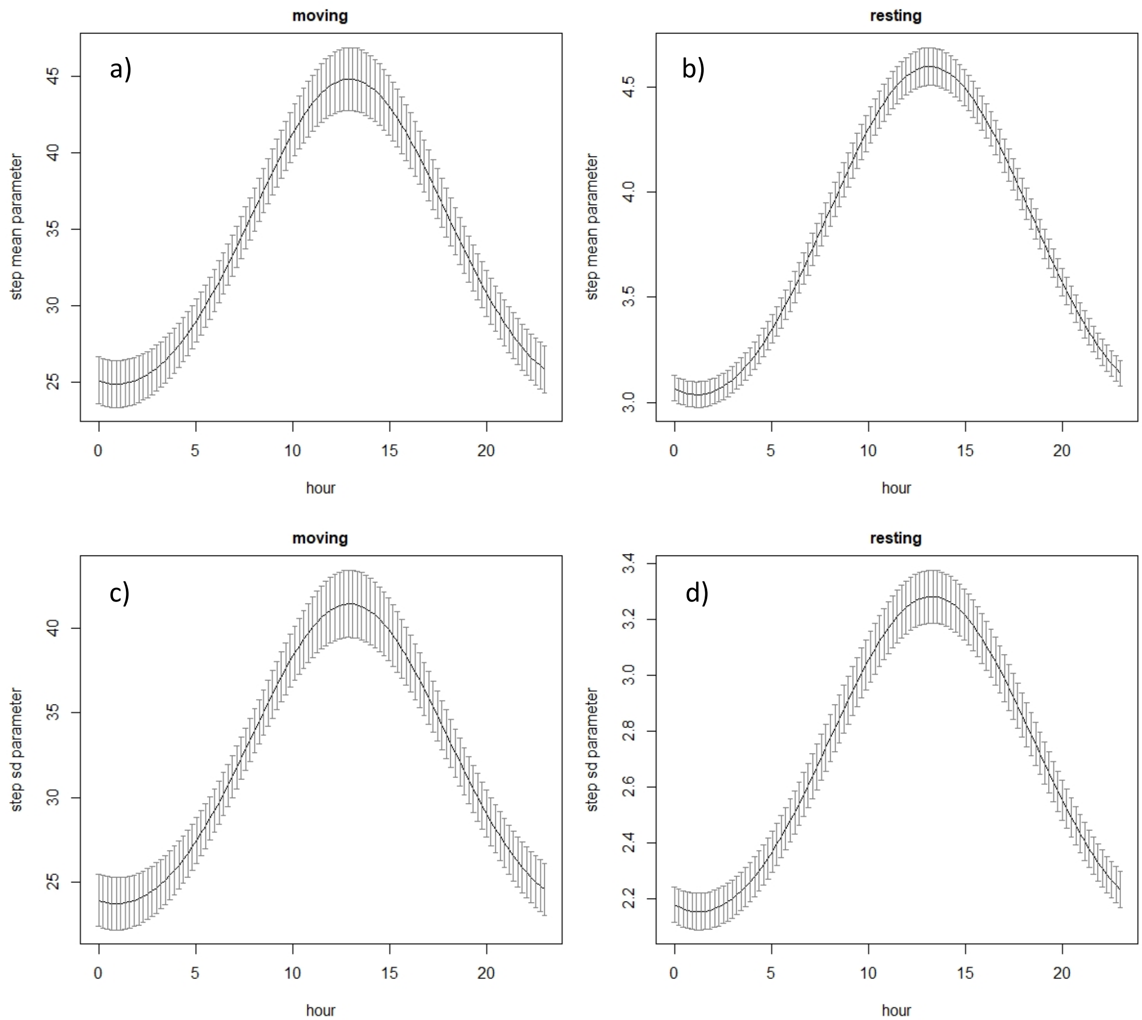


**Figure 10.** Diurnal variation in mean steplength during moving (a) and resting (b) movement states and diurnal variation in standard deviation (SD) of steplengths during moving (c) and resting (d) movement states. Error bars are 95% confidence intervals. Mean steps and SD are in meters.

Tortoises exhibited overlapping space use between movement states, alternating between the localized resting state and longer travelling behaviors during the moving state (Figure 11, Appendix A). At any given time tortoises were most likely to stay in the movement state that they were already within (e.g., a resting tortoise tended to keep resting), but when switching between movement states tortoises were more likely to switch from the resting state to the moving state during midday (e.g., 9:00 – 13:00) than during the evening, night, or morning (Figure 12). When downshifting from moving to resting, tortoises were more likely to do so in the afternoon (e.g., 13:00 – 17:00; Figure 10).

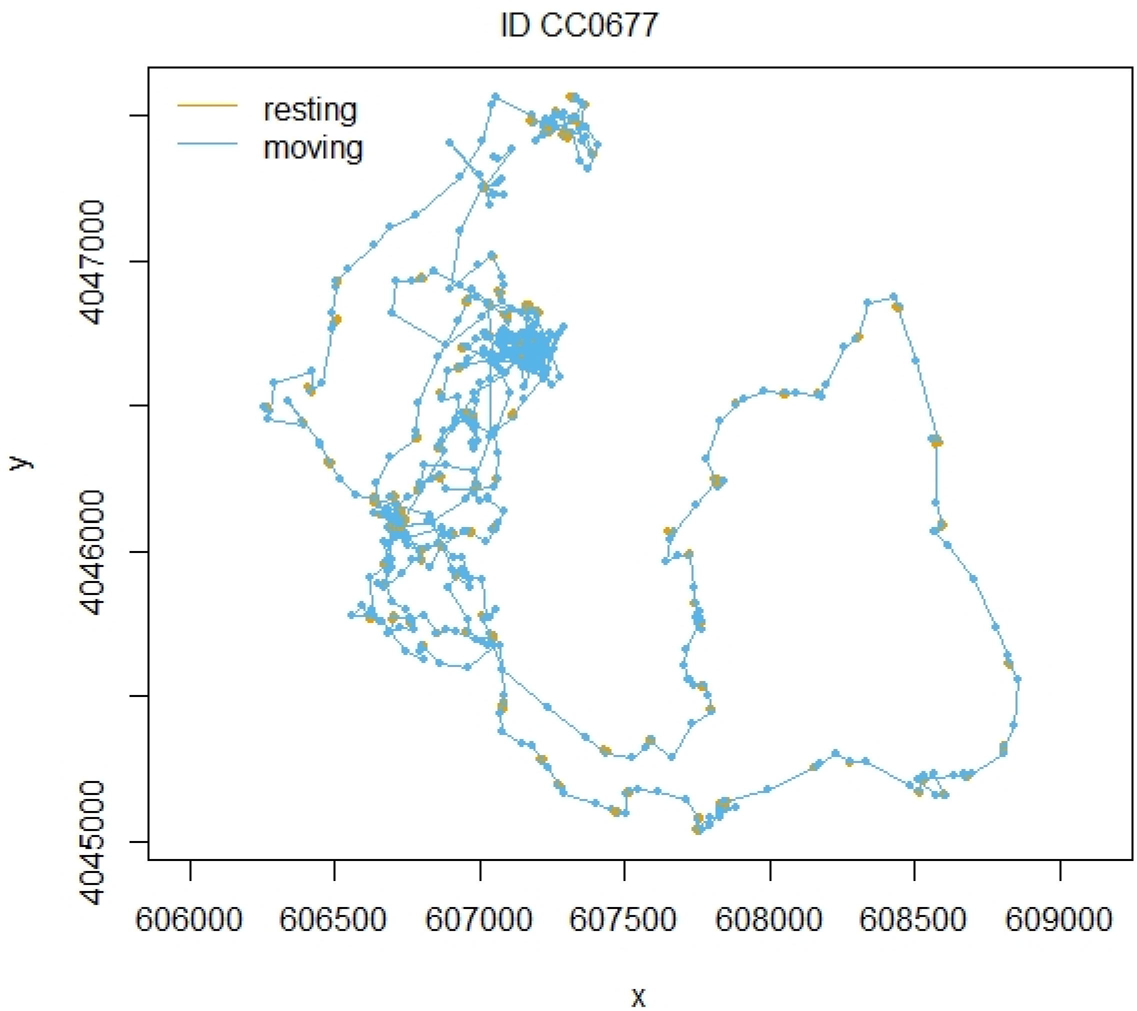


**Figure 11.** Assignment of GPS locations to one of two mutually exclusive movement states, resting vs. moving, for a single Mojave desert tortoise (ID CC0677). X and y are UTM coordinates (NAD83, Z11N). See Appendix A for movement state assignment for locations from all tortoises.

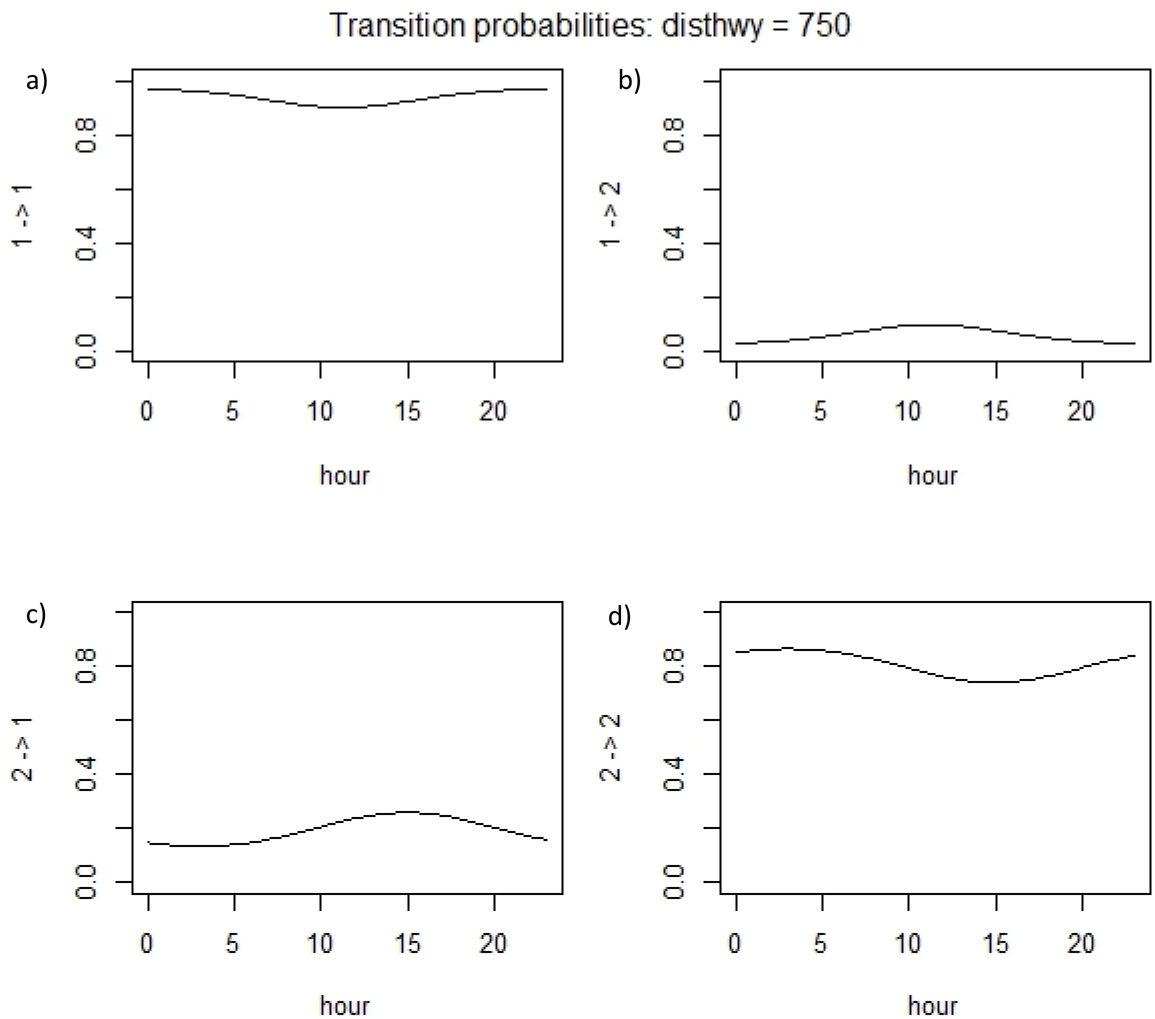


**Figure 12.** Diurnal variation in probabilities of remaining in a resting state given currently being in a resting state (e.g., 1 → 1; a), transitioning from a resting to a moving state (e.g., 1 → 2; b), transitioning from a moving to a resting state (e.g., 2 → 1; c), or remaining within a moving state (e.g., 2 → 2; d) when a tortoise was the average observed distance from the highway (disthwy = 750 m).

Using this two-state time-of-day model as a null baseline biological movement model, there was strong evidence ($\Delta$AIC = 50.3) that transitions between moving and resting states, but not movement behaviors within those states, were non-linearly influenced by how close the tortoise was to the highway. (Table 5). As tortoises were nearer to the highway, those that were resting were more likely to continue resting and less likely to switch from a resting to a moving state ($\beta = 0.23$, 0.14 - 0.32; $p < 0.05$) and conversely, when tortoises were in a movement state and closer to the highway they were more likely to switch to a resting state ($\beta = -0.12$, -0.21 - -0.03; $p < 0.05$) and less likely to continue moving (Figure 13). The highway effect was non-linear, with most of the highway effect on transition rates occurring within 0 - 1,000 m of the highway (Figure 13).

**Table 5.** Model selection results for models evaluating the influence of U.S. Highway 95 on movement behaviors within, and transition rates between, resting and movement states as a function of linear and non-linear (log) distance to the highway. AIC is an information criterion to select the most parsimonious model for the data from among the candidate set. The variable 'weight' is the relative weight of evidence in favor of a given model as being the best model from within the candidate set. All models included within-state (time of day) + transition (time of day) terms for the base biological movement model (Table 4).

| Model | AIC | DeltaAIC | weight |
|---|---|---|---|
| Transition (log(dist highway)) | 375643.6 | 0.0 | 0.96 |
| Within-state (log(dist highway)) + Transition (log(dist highway)) | 375650.0 | 6.4 | 0.04 |
| Transition (dist highway) | 375685.8 | 42.2 | 0.00 |
| Baseline biological movement | 375693.9 | 50.3 | 0.00 |

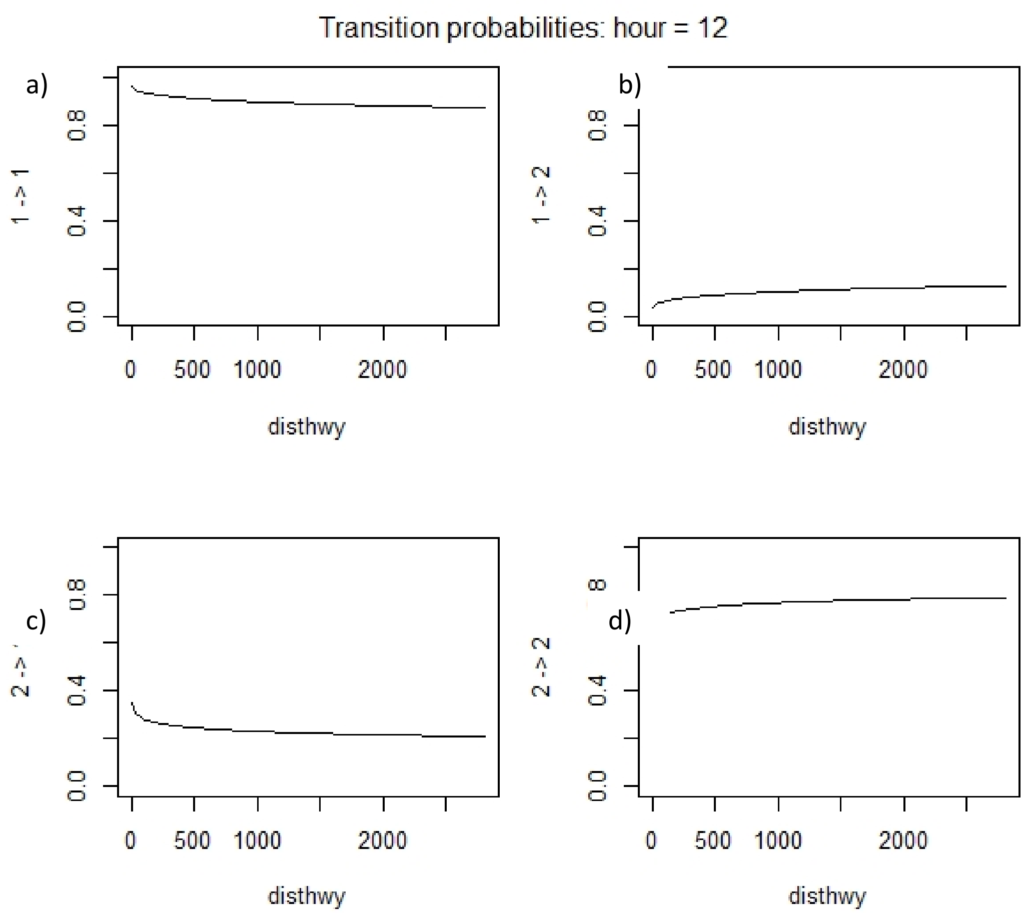


**Figure 13.** Spatial variation in probabilities of remaining in a resting state given currently being in a resting state (e.g., 1 → 1; a), transitioning from a resting to a moving state (e.g., 1 → 2; b), transitioning from a moving to a resting state (e.g., 2 → 1; c), or remaining within a moving state (e.g., 2 → 2; d) at 12:00 pm as a function of the natural log of the distance to U.S. Highway 95 in northwestern Clark County, Nevada.

## DISCUSSION

Plots along Hwy 95, with large box culverts, had desert tortoise abundance consistently and significantly higher on the south side of the highway than the north side, for all plots that had any tortoises. This is consistent with the hypothesis that the culverts were not promoting admixture of desert tortoises across the highway. Along Hwy 93, with cylinder culverts, tortoise abundance showed no consistent variation along or on either side of the highway, which is consistent with the hypothesis that the road was not acting as an impermeable barrier. However, because the study was a single snapshot of tortoise abundance after fencing and culvert construction (i.e., not a Before-After Control-Impact design), our ability to make inferences via mark-recapture alone is inherently limited. It is possible that for Hwy 95, populations are free to mix but habitat quality north of the highway is sufficiently lower than south of the highway that resident populations are denser to the south. Although possible, this is probably unlikely, simply given the close proximity of plot-pairs and thus presumed similarity of vegetative and soil conditions. It is also possible that for Hwy 93, the road is an impermeable barrier and that apparent random variation in tortoise abundance is solely a function of local habitat conditions.

The use of culverts to cross highways was positively related to the disparity in desert tortoise density on either side of the highway, but not to overall high density. This indicates that the function of culverts for connectivity is not simply a matter tortoise encounters of the culverts, but rather that tortoises use the culverts as dispersal corridors when disparity in density is high.

There was overwhelming support for a two-state movement model versus a single state model, whereby tortoises exhibited movement behaviors representative of two mutually exclusive 'states'. When resting tortoises exhibited short steplengths with strongly recursive turning angles (i.e., turning back) whereas when moving tortoises exhibited long steplengths that on average were not in consistent directions. Steplength distances within either movement state were also strongly non-linear with respect to time of day.

Crossing rates across U.S. Highway 95 for GPS-tagged tortoises were very low, with only two observed crossings across two out of 1,298 monitored tortoise-days. This may have been explained by the observed indirect effect of the highway on transition rates between resting and moving states. When closer to the highway, tortoises tended to stay resting if already resting and were more likely to drop into the resting state if already moving. Thus although tortoises here and elsewhere are known to use culverts to cross highways (Boarman et al. 1998, S. Cambrin unpublished data), the indirect mechanism of the highway on the movement state of tortoises may reduce the likelihood that tortoises encounter and use the culverts.

The effect of roads have been documented previously for Mojave desert tortoises. Direct mortality is a known concern, with higher numbers of dead tortoises and lower numbers of live tortoises encountered near roads with increasingly higher traffic volumes (Nafus et al. 2013). Concomitantly, burrow density is lower along high traffic roads and tortoise sign density is higher further from roads (Nafus et al. 2013). Aside from direct mortality, subadult Mojave desert tortoises have been observed moving less when near minor roads, yet contrastingly moving more when near roads that are fenced (Sadoti et al. 2017). In our study, the influence of direct mortality is expected to be limited to legacy effects, as the highway has been fenced and regularly maintained for > 6 years prior to data collection for this study. For example, legacy effects could include past direct impacts on population structure that are currently observable, such as past mortality of larger, older tortoises and therefore current skewing of age biases towards juveniles and subadults (Nafus et al. 2013). In contrast, the current effects of the highway should be limited to indirect impacts, such as impacts on movement, space use, and behavioral results observed here. These might be driven primarily by the traffic noise and/or vibrations along this major U.S. highway, a hypothesis which has been proposed but is currently untested (Peaden et al. 2015).

A road-induced behavioral shift in the basic movement state occupied by desert tortoises could have implications for population growth, even in the absence of direct mortality (Nafus et al. 2013). During periods of drought, mortality of Mojave desert tortoises is significantly elevated (Longshore et al. 2003, Lovich et al. 2014). In part to counteract the reduction of available food and water content during drought, tortoises reduce activity levels to conserve water energy (Duda et al. 1999). However, if drought conditions are not present, behavioral inducement to reduce time spent foraging, as observed here via proximity to the highway, could reduce foraging during critical 'good times' near roads with concomitant increases in mortality. Further research into the mechanisms behind behavioral state switching near roads may identify ways to ameliorate the negative impacts. This may occur in spite of evidence that tortoises are not averse to residing in and using habitat along fenced roadways (Hromada et al. 2020).

Culverts have been shown to be used as crossing structures by Mojave desert tortoises under roads and highways. However, desert tortoises are more likely to switch to and stay in resting states when nearer to the highway, meaning that they are also less likely to encounter and use culverts. Thus culvert placement might be optimized by facilitating noise and/or vibration reduction in the surrounding portions of the highway, either through potential technological design or alignment with local geology, to reduce the indirect road impact reducing culvert use. Further, culvert use was positively associated with the disparity in tortoise abundance on either side of the highways, suggesting that culverts best function as dispersal corridors rather than as regular travel corridors.

**Key management take-aways:**

- Abundance of tortoises differed markedly on either side of highway culverts.
- Culverts did not appear to equalize tortoise abundance across the highway.
- Culverts did function as connectivity conduits, supported by camera and GPS data.
- The connectivity benefit of culverts was positively related to disparity in abundance on either side of the highway, suggesting benefits related to dispersal rather than regular movement.
- Tortoises were more likely to switch to, and stay in, resting movement states when nearer to the highway.
- Behavioral state switching and maintenance may be a mechanistic effect behind the indirect impact of highways on tortoise populations.
- Future culverts would be best placed in locations with disparate abundances on either side of the road, with the recognition that indirect road effects may reduce, but not eliminate, the connectivity benefits of culverts.

## LITERATURE CITED

Boarman, W. I., M. L. Beigel, G. C. Goodlett, and M. Sazaki. 1998. A passive integrated transponder system for tracking animal movements. Wildlife Society Bulletin 26:886-891.

Duda, J. J., A. J. Krzysik, and J. E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. Journal of Wildlife Management 63:1181-1192.

Gray, M. E., B. G. Dickson, K. E. Nussear, T. C. Esque, and T. Chang. 2019. A range-wide model of contemporary, omnidirectional connectivity for the threatened Mojave desert tortoise. Ecosphere 10:e02847.

Hromada, S. J., T. C. Esque, A. G. Vandergast, K. E. Dutcher, C. I. Mitchell, M. E. Gray, T. Chang, B. G. Dickson, and K. E. Nussear. 2020. Using movement to inform conservation corridor design for Mojave desert tortoise. Movement Ecology 8:38.

Lecomte, J., K. Boudjemadi, F. Sarrazin, K. Cally, and J. Clobert. 2004. Connectivity and homogenization of population sizes: an experimental approach in *Lacerta vivipara*. Journal of Animal Ecology 73:179-189.

Longshore, K. M., J. R. Jaeger, and J. M. Sappington. 2003. Desert tortoise (*Gopherus agassizii*) survival at two Eastern Mojave desert sites: death by short-term drought? Journal of Herpetology 37:169-177.

Lovich, J. E., C. B. Yackulic, J. Freilich, M. Agha, M. Austin, K. P. Meyer, T. R. Arundel, J. Hansen, M. S. Vamstad, and S. A. Root. 2014. Climatic variation and tortoise survival: has a desert species met its match? Biological Conservation 169:214-224.

McClintock, B. T., J. M. London, M. R. Cameron, and P. L. Boveng. 2017. Bridging the gaps in animal movement: hidden behaviors and ecological relationships revealed by integrated data streams. Ecosphere 8:e01751.

Michelot, T., R. Langrock, and T. A. Patterson. 2016. moveHMM: an R package for the statistical modelling of animal movement data using hidden Markov models. Methods in Ecology and Evolution 7:1308-1315.

Nafus, M. G., T. D. Tuberville, K. A. Buhlmann, and B. D. Todd. 2013. Relative abundance and demographic structure of Agassiz's desert tortoise (*Gopherus agassizii*) along roads of varying size and traffic volume. Biological Conservation 162:100-106.

Peaden, J. M., T. D. Tuberville, K. A. Buhlmann, M. G. Nafus, and B. D. Todd. 2015. Delimiting road-effect zones for threatened species: implications for mitigation fencing. Wildlife Research 42:650-659.

Sadoti, G., M. E. Gray, M. L. Farnsworth, and B. G. Dickson. 2017. Discriminating patterns and drivers of multiscale movement in herpetofauna: the dynamic and changing environment of the Mojave desert tortoise. Ecology and Evolution 7:7010-7022.

Valentine, J. 2021. Desert tortoise connectivity across roadways, project number: 2015-ECOCENT-1580B. Final Report.