Final Report

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DESERT TORTOISE CONNECTIVITY SOLUTIONS MODELING

Prepared for the Desert Conservation Program, Clark County, Nevada

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Introduction

Desert Tortoise connectivity

The Mojave desert tortoise (*Gopherus agassizii*) is listed as threatened and is afforded protection under Federal and State endangered species provisions throughout its range in the Mojave Desert and parts of the Sonoran Desert in Nevada, California, Utah, and Arizona (U.S. Fish and Wildlife Service 1994 and 2011). One of the recovery actions listed in the 2011 Revised Recovery Plan for the Mojave Population of the Desert Tortoise is to determine the importance of corridors and physical barriers to desert tortoise distribution and gene flow (Recovery Action 5.5). In areas subject to anthropogenic pressures, corridors improve opportunities for individual contact and gene flow. It is important then to determine attributes of corridor suitability (e.g. size in the context of suitable habitat and disturbance levels), and to examine how linear barriers may impede otherwise connected habitat. Corridors are needed to allow movement between habitat patches, prevent genetic isolation, and ultimately to ensure persistence of the species.

High levels of gene flow and isolation-by-distance (IBD) play an important role in genetic connectivity for tortoises across their range (Hagerty and Tracy 2010; Murphy et al. 2007). However, IBD does not account for landscape features (e.g. mountains, playas, anthropogenic disturbance) that may influence gene flow. Support for alternative models acting in conjunction with IBD, such as isolation-by-resistance has been found on a broad-scale with mountains and valleys limiting gene flow (Hagerty et al. 2011; Sanchez-Ramirez 2018), and at a finer spatial scale with roads acting as barriers (Dutcher et al. 2020; Latch et al. 2011). Roads are associated with high tortoise mortality and reduced abundance ranging from 0.2 to 4 km from the road, depending on traffic volume (Boarman and Sazaki 2006; Nafus et al. 2013; Peaden et al. 2015; von Seckendorff Hoff and Marlow 2002). Tortoise persistence may rely heavily on the ability to disperse across the landscape (Edwards et al. 2004) and road fencing tied in with underground hydrological culverts may ease mortality rates and allow for gains in connectivity (Boarman et al. 1997; Boarman and Sazaki 2006; Ruby et al. 1994).

Because landscape changes that impact populations, positively or negatively, are associated with a time lag measured in generations (Landguth et al. 2010) and tortoises are long lived, detection of demographic and genetic shifts often occurs well after the landscape has been altered. Long-term monitoring has revealed that tortoise populations continue to decline even within most protected areas, likely influenced by anthropogenic habitat use (Allison and McLuckie 2018; Averill-Murray 2021). Declines in large tortoises may reflect human disturbance (Corn 1994) and are potentially problematic as survival of large adults, especially females, strongly impacts population growth (Doak et al. 1994). Increasing development pressures across tortoise habitat continue to increase habitat loss and fragmentation while highlighting the need to maintain connected habitat (Averill-Murray et al. 2013).

The University of Nevada, Reno (UNR) recently completed a project looking at the 17 most crucial areas in Clark County, Nevada for desert tortoise connectivity and determined that seven of those areas currently have a low connectivity potential or fail to maintain connectivity into the future based on future development projections (Dutcher et al. 2019). The project used available software applications to simulate tortoise population genetics through time, but the models were limited in scope and realism due to memory and parameter limitations. Two important limiting features were the inability to model overlapping generations, which is important toward understanding the potential for genetic impacts through time, and modeling populations at the scale of the likely habitat patches, but with sufficient resolution to represent realistic barriers to movement.

This project uses individual-based modeling (aka agent-based) to attempt to provide a more realistic approach to understanding the potential for tortoises to maintain connectivity in light of disturbance on landscapes associated with urbanization and other anthropogenic impacts and features. We seek to address these questions by modeling connectivity of tortoise populations among areas in fragmented habitat to better understand the possible influences of anthropogenic disturbance on genetic connectivity and population demographics of desert tortoises in areas within Clark County, Nevada differentially impacted by anthropogenic activities and barriers to movement by modeling movement, mating and demographics, and population genetics.

Urbanization and human population growth

The Intergovernmental Panel on Climate Change used a range of radiative forcing levels (values that reflect the change in energy flux) to generate four Representative Conservation Pathways (RCPs) with levels from 2.6 to 8.5. A positive radiative forcing value indicates the earth is receiving more incoming energy from sunlight than is reflected and signifies warming. Analysis described by van Vuuren et al. (2012) noted little relationship between radiative forcing levels and human population, rather forcings on a global scale are heavily influenced by historic and future emission levels in conjunction with already rising temperatures along with land use change (i.e. expansion of agriculture and urbanization). Subsequently, a research group focused on trajectories for human development and global environmental change established five global shared socioeconomic pathways (SSPs) scenarios (van Vuuren et al. 2017). The five SSPs reflect relative emissions resulting from anthropogenic change caused by human population growth and land use at a continent/country level. In the United States much of the potential future change in emissions is a direct result in projected human population growth resulting in increased urbanization. Increased urbanization (residential, commercial, or industrial) has the potential to influence the quantity and quality of habitat available for native species. Over the past three decades the desert southwest of the United States has seen some of the largest increases in human population making the region an important focus area for national future population growth and urbanization analyses (Theobald 2013).

Landscape genetics and demography

Genetic diversity allows populations to withstand a wider range of environmental changes, including climatic extremes (Bijlsma and Loeschcke 2005). Genetic variation is introduced into populations by genetic mutations and dispersal. Therefore, genetic diversity statistics are useful in estimating dispersal ability through gene flow, or connectivity. When dispersal distance is less than the geographic distance between individuals in a continuous population individuals mate with those closer to them, producing a naturally occurring stepping stone pattern of connectivity (IBD, Wright 1943; Kimura and Weiss 1964). The spatial genetic structure produced by IBD may confound inference of gene flow where habitat loss and fragmentation have disrupted the landscape because contemporary genetic patterns may not be as apparent as historic patterns due to time lags in detection (Leblois et al. 2006).

Urbanization has been associated with increased population genetic structure across taxa (Barr et al. 2015; Hagell et al. 2013; Richmond et al. 2016; Vignaud et al. 2013). Habitat loss and fragmentation reduce population sizes and can impede connectivity (Ewers and Didham 2006; Fahrig 2003; Haddad et al. 2015; Hand et al. 2014). Stronger signals of population genetic structure and loss of genetic diversity may emerge as breeding groups become smaller and more isolated from one another (Richardson et al. 2016). Loss of genetic diversity is predicted to increase extinction risk and is proportional to population size; therefore, small populations typically exhibit less genetic diversity than large populations as the result of genetic drift, or non-random mating (Frankham et al. 2009). Using genetic and demographic methods enhances our understanding of functional landscape connectivity by combining gene flow estimates with demographic rates related to dispersal, mortality, and reproduction (Lowe and Allendorf 2010).

Study approach

Given the accelerated pace of habitat disturbance in Mojave desert tortoise habitat and long generation times for the species, real time study of current and planned impacts is challenging. Using resistance surfaces from large areas across Clark County, Nevada we simulated gene flow across complex landscapes to evaluate multiple barrier scenarios. We incorporated areas predicted to fail to maintain genetic connectivity based on low connectivity index scores determined by previous work (Desert Tortoise Connectivity Modeling, 2015-UNR-1580A, Dutcher et al. 2019). Simulations were run forward-in-time for 100 years using realistic parameters for movement, mating, and mortality derived from empirical studies. Demographic and genetic patterns were predicted from simulation output to better understand the consequences of specific actions. Our overarching goals were two-fold:

1. Develop a model suitable for appropriate landscape scale analyses
2. Use the model to discern which factors most affect connectivity at specific locations and which available solutions best alleviate the stress of human land use

Materials and Methods

Human population forecast and future land use

The SSP describing maximum anthropogenic disturbance and environmental change was selected to represent the extreme human population projection (van Vuuren et al. 2017). This approach was taken to examine whether we could detect substantial genetic and demographic changes at the highest levels. The high growth SSP provided the foundation to parameterize human population for our urban growth model to year 2100. The SSP was implemented at the extent of Clark County, Nevada and centered on the Las Vegas metropolitan area. Urban growth futures share three fundamental informational pieces driving the quantity and distribution of future urbanization:

1. Human population forecasts – based on an estimate of population change from the starting population in 2010 to 2100
2. Buildable lands identification – current land use restrictions dictate where anthropogenic development activities may occur
3. Attractors for urban development – a method using established disturbance to select one parcel of land over another for development

Human population forecasts used a starting population from 2010, with an estimated 2.68 people per household within our study region (U.S. Census Bureau 2010). Human population data taken at the U.S. Census tract level were lumped into four population centers in Southern Nevada: Las Vegas, Boulder City, Pahrump, and Mesquite. U.S. population size estimates using SSP for years 2050 and 2100 (KC and Lutz 2017) fit a 2nd order polynomial linear model determined using R v.4.1.1 (R Core Team 2021). Yearly population increases from 2011 to 2100 were calculated using a rate curve derived from the linear model (Figure 1). The population component of the urban growth model was parameterized with the 2010 starting population, average number of people per household, and urbanized footprint per household for Las Vegas, Nevada (Trammell et al. 2018).

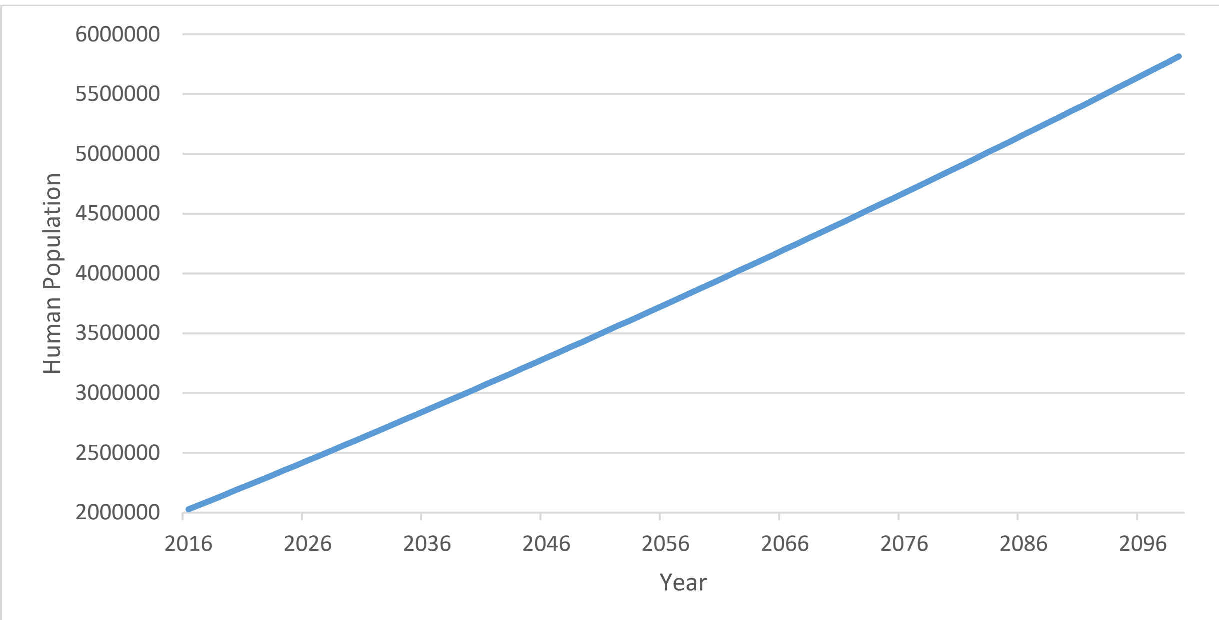


Figure 1 - Projected human population increase for the Las Vegas, Nevada metropolitan area from 2016 to 2100.

Buildable land identification incorporated areas where land management practices do not prohibit the building of structures and natural processes do not inflate costs associated with construction. Five components comprised buildable land identification:

1. Slope < 20%
2. Non-urban lands
3. Not open water or wetland

4. Private land or land owned by the U.S. Bureau of Land Management in Nevada
5. Land not designated with a conservation or preservation category

The five components were combined into a single Geographic Information System (GIS) layer depicting areas available for urban development using ArcGIS© v.10.7.1 (Figure 2). A 10-meter resolution digital elevation model (DEM) served as the base for calculating slopes less than 20% (U.S. Geological Survey 2019). Existing urban lands, open water, and wetlands were extracted from the Gap Analysis Program (GAP) and LANDFIRE National Terrestrial Ecosystems dataset (U.S. Geological Survey 2011). We accessed a land management and ownership database to determine land status (U.S. Bureau of Land Management 2019). Conservation or preservation areas were identified using the GAP Protected Areas Database (U.S. Geological Survey 2018) and eliminated from consideration as developable. Proposed areas of environmental concern and off-highway vehicle (OHV) locations were included as unbuildable.

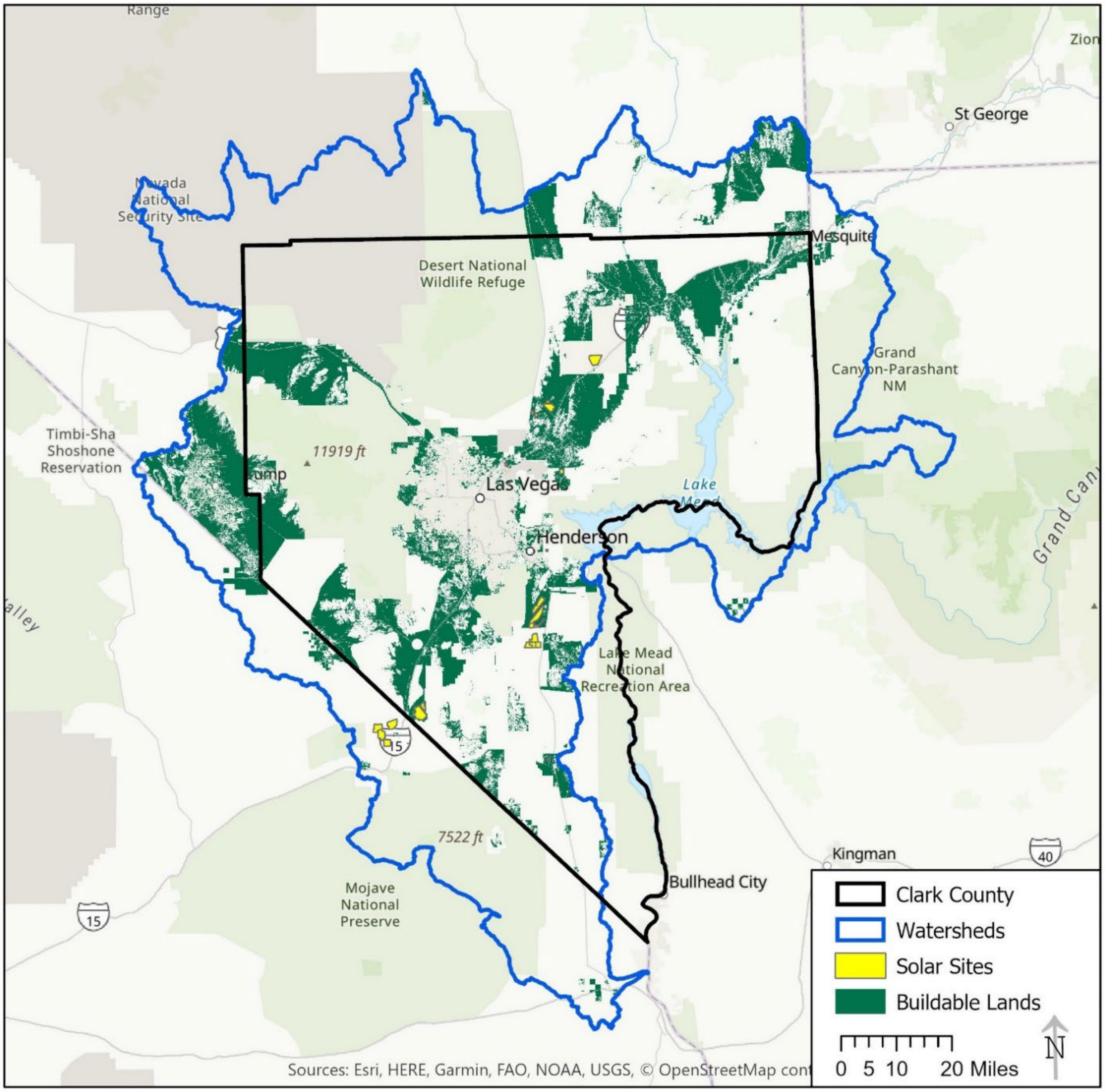


Figure 2 - Areas where development is allowed to occur (buildable lands) and existing industrial scale solar sites are located within the watersheds in the study area.

Attractors for urban development included existing urbanization and major roads, which we extracted using the GAP/LANDFIRE National Terrestrial Ecosystems dataset (U.S. Geological Survey 2011). A Euclidean distance function was then executed for all non-urbanized areas and used to rank attractiveness for development where the closer a buildable piece of land was to existing urban development the more likely it was to become developed in the future. The Jean

Airport in the Ivanpah Valley on the Nevada-California border was estimated to be completed by 2040 and added as an attractor for future urbanization. Locations within the proposed Jean Airport boundary and Noise Containment Area were set to attract new urbanization within those areas first. Additional developments currently in the study region include industrial scale solar (Figure 3). Approved and proposed industrial scale solar project locations were obtained from U.S. Bureau of Land Management and incorporated into future land use with construction assumed to be finished by 2030 (approved) and 2040 (proposed; Figure 3).

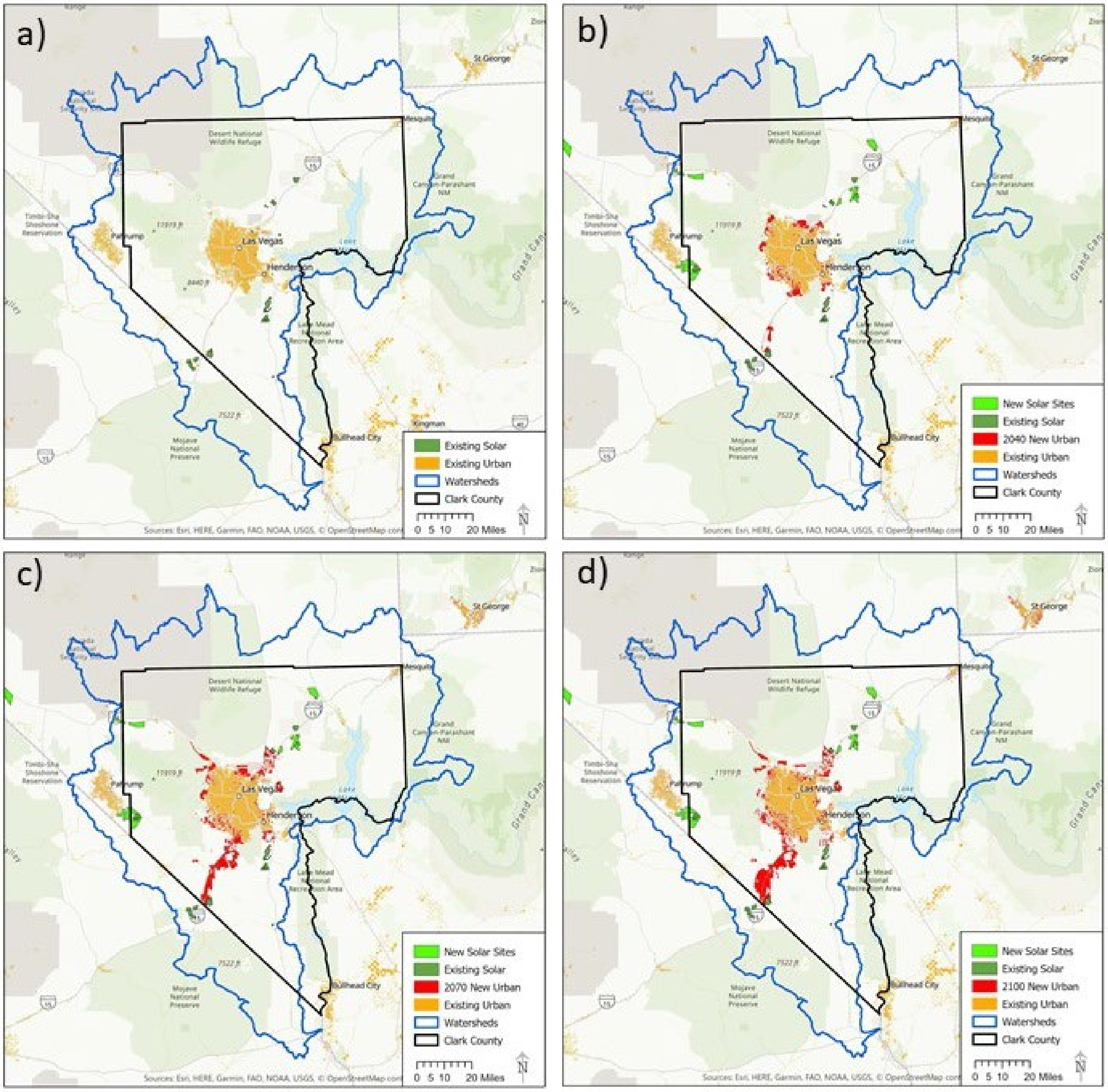


Figure 3 - Current urbanization and future land use change forecasts in Clark County, Nevada for years a) 2021, b) 2040, c) 2070, and d) 2100.

Road and railroad GIS data layers were obtained from the U.S. data repository (catalog.data.gov) which originated from Tiger/Line files at the U.S. Census Bureau. Roads were classified as primary, secondary, or local by road name (e.g. Interstate 15) and the average annual daily traffic (AADT) volumes for primary roads were assigned using the Nevada

Department of Transportation's Trina dataset. Primary roads were also assigned a permeability value based on their AADT values with heavily trafficked roads (i.e. interstates) considered not passable and minor roads (e.g. Goodsprings and Nipton Roads) considered the most passable. Culverts under primary roads were included with attribute information indicating tortoise ability to use each culvert as a crossing structure (provided by Clark County, Nevada). Each culvert was assigned a value ranging from 1 to 5, from the most passable culverts to the least: 80% passable (1), 60% passable (2), 40% passable (3), 20% passable (4), not passable (5). Three scenarios were identified for culvert use by desert tortoises in the Ivanpah Valley: Culvert 1 assumed the status quo of the assigned values and that culverts were not obscured by fencing; Culvert 2 assumed culverts located in a 4 to 5-mile stretch of Interstate 15 (I-15) between the proposed Jean Airport Noise Containment Area and the southwestern edge of the Las Vegas metropolitan area were modified to facilitate desert tortoise movement and were assigned a score of 80% passable. The final scenario, Culvert 3, considered the current state, where many of the culverts are not tied into the tortoise fencing, and thus cannot be used by tortoises, despite their otherwise sufficient condition.

Study areas and digital representation

Six study areas were identified across Clark County, Nevada for our analyses (Figure 4). Study area boundaries reflected the combination of watershed units delineated as part of the Watershed Boundary Dataset produced by the U.S. Geological Survey (2021). These areas were chosen to achieve maximal areas of relatively discrete tortoise habitat that likely have minimal gene flow between them, that were also tenable for analysis given computational limitations.

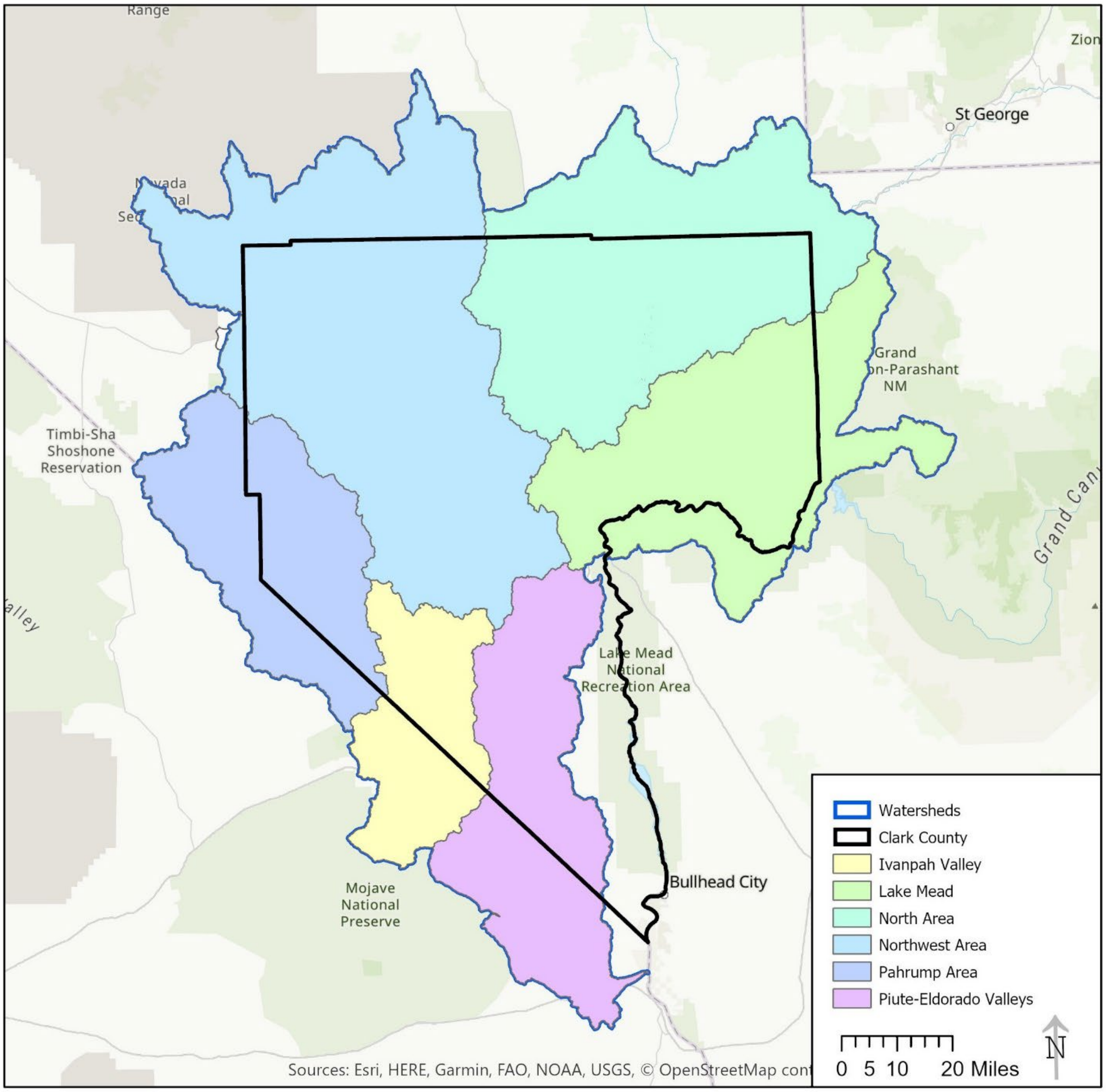


Figure 4 - Study areas within Clark County, Nevada used in our analyses. The six study areas are: Ivanpah Valley, Lake Mead area, North area, Northwest corridor, Pahrump area, and Piute-Eldorado Valley.

A least-cost path algorithm was used to simulate movement on landscape surfaces (Dijkstra 1959). Modeling movement across landscapes can be a computationally expensive process. Using agent-based models adds to the expense because the least-cost path algorithm runs for each individual at each time step in the simulation. Computational intensity also depends on the resolution of the raster representing the landscape, with smaller cell sizes increasing computation time, and RAM (random access memory) needed to store raster layers. This results in a trade-off between run time, performance, and landscape detail. To minimize this trade-off, we developed a quadtree data structure and created an R package (*quadtree* package v.0.1.6, Friend 2021) to allow simulations to remain computationally tractable with fine-scale spatial details. Unlike rasters, quadtrees can have variable cell sizes, with a minimum of 30 m in our simulations (Figure 5). This allowed heterogeneous areas to be represented by smaller cells and homogeneous areas by larger cells (Samet 1984). Necessary landscape information was retained by representation at a fine-scale (e.g. areas along major roads that contain culverts) while only causing a small increase in overall computation time (van Bemmelen et al. 1993).

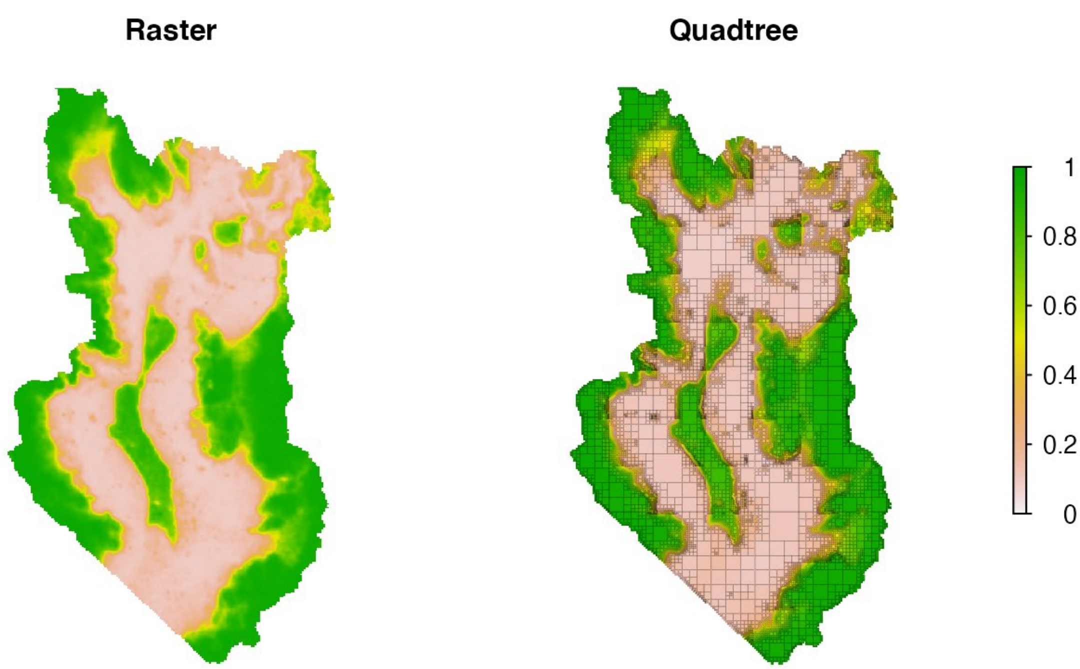


Figure 5 - The raster and quadtree representations of the desert tortoise habitat model for the Ivanpah Valley study area along the Nevada-California border.

Initial genetic data

Genetic samples collected within the Ivanpah Valley along the Nevada-California border were used to create a dataset of initial genotypes amplified at 20 variable microsatellite loci (Figure 6). Microsatellite loci are generally not influenced by natural selection and mutations are allowed to accumulate without cost, making them model markers for understanding processes such as gene flow, migration, and dispersal (Holderegger et al. 2006). The initial genetic data were tested for departures in Hardy-Weinberg equilibrium (HWE, allele frequencies indicative of random mating) using an exact test with Bonferroni correction (adjusted p-value = 0.003 for α = 0.05) and found to be in equilibrium. Missing alleles in the initial dataset were replaced by mean values. Samples were randomized and simulated forward-in-time using a burn-in period of 100 years to create seed genotypes on a habitat surface for each location evaluated.

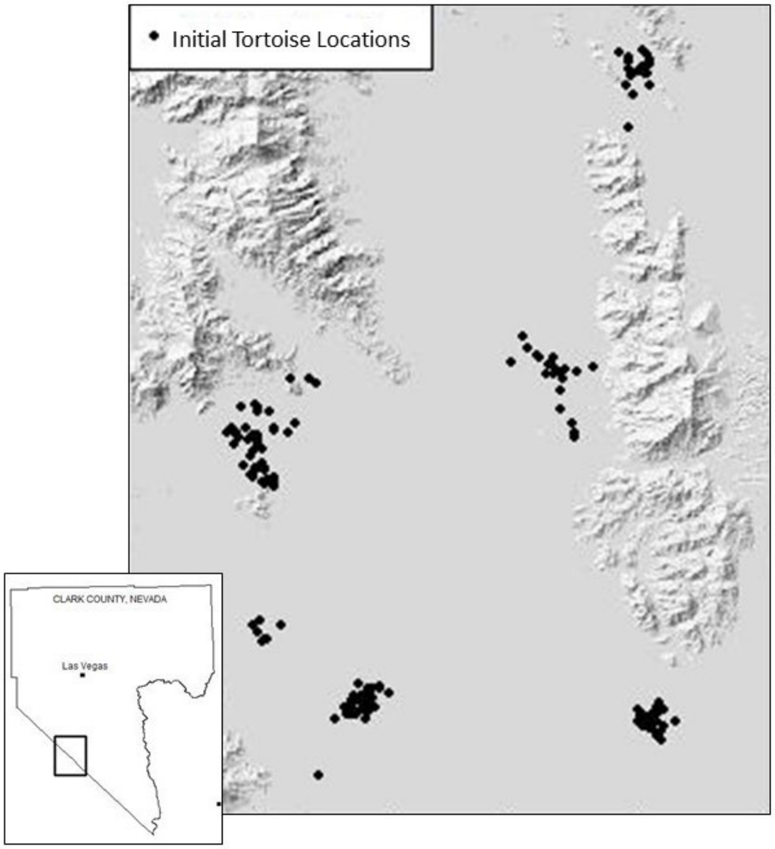


Figure 6 - Initial locations of genetic samples collected within the Ivanpah Valley, along the Nevada-California border, used to create genotypes for landscape scenario simulation models. Adapted from Dutcher et al. 2020.

Forward-in-time simulation framework

An individual-based forward-in-time modeling framework was created in R to construct simulations accounting for variable barrier configurations and urban growth through time. This consisted of generating an initial random population of tortoises for each study area, that were then established using a burn-in run of 100 years with a simulated habitat only cost landscape (habitat values = 1) to allow for the population density to adjust to the influences of local habitat condition and spatial habitat arrangement. Simulations were then run for 100 annual cycles for each area and configuration, and included dispersal, breeding, and mortality for each year. Each tortoise was tracked individually throughout its lifetime. Genotypes, movements, and demographic parameters were recorded for each year of the simulation.

Movements were simulated to represent an annual displacement for each tortoise. These dispersal movements were based on the yearly home range shift of resident radio-telemetered tortoises tracked for multiple years at eight sites in the Mojave Desert in Nevada (previously reported in Nussear et al. 2012; Drake et al. 2012 and 2015; Sah et al. 2016; Hromada et al. 2020): Bird Spring Valley (n=120), Coyote Springs (n=118), Halfway Wash (n=47), Lake Mead (n=9), McCullough Pass (n=20), Piute Valley (n=129), Stateline Pass (n=11), and California: Fort Irwin (n=263). For each iteration of annual dispersal, a random bearing for direction of movement was drawn, and a random number between 0 and 1 was used to find the closest corresponding home range shift distance from a probability distribution function derived from the movement dataset (Figure 7). Movement cost was considered to be the inverse of habitat suitability (in the absence of anthropogenic disturbance), such that areas of high suitability were "easier" for tortoises to move through than those of lower suitability (e.g. rough mountainous areas or vast dry lakes that are not typically considered habitat). A new destination point was calculated using the randomly generated bearing and distance from the distribution discussed above. A movement path was then calculated from the animal's current location to the new location using a least-cost path using the *lcp_finder* and *find_lcp* functions in the *quadtree* package. Due to irregularities on the landscape (e.g. the least-cost path traces around an obstacle), it was possible that the least-cost path was longer than the desired displacement distance, as the movement path varied to avoid obstacles or poor habitat (Figure

8). An accumulation cost was calculated to stop movement at the location where the selected cost adjusted displacement distance was achieved. As the movement model placed all tortoises in the center of the cost surface quadtree grid cell, all final locations were selected at random within an ellipse formed between the last two points (note - this inadvertently created unintended barrier crossovers in barrier runs). Tortoises that dispersed into completely unsuitable habitat (e.g. somehow ending up on a road or off the edge of the map) were considered mortalities for that year.

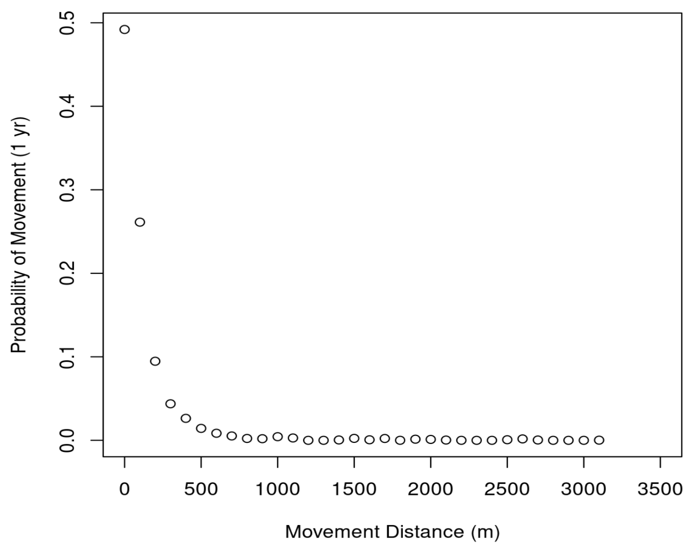


Figure 7 - Probability of annual movement distances calculated from annual kernel home range centroid shifts for 717 desert tortoises at eight Mojave Desert sites.

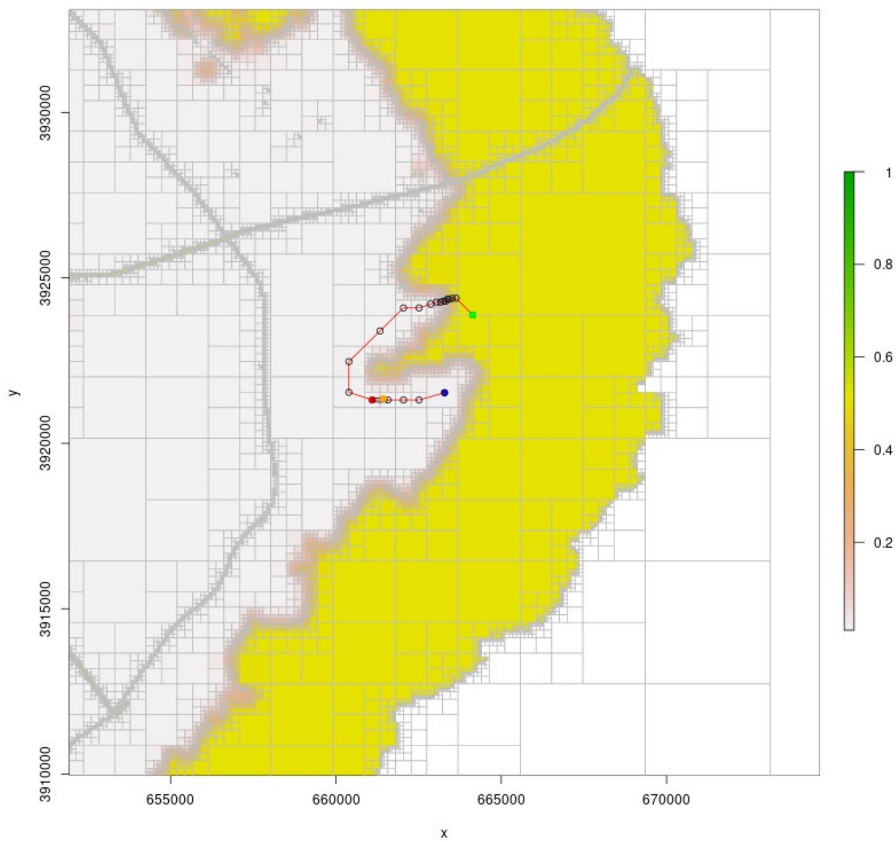


Figure 8 - Least-cost path example that caused an animal to travel beyond the displacement distance with movement halted at the appropriate distance. The blue dot represents the origin, the green dot denotes the random destination, line segments indicate the least-cost path, the red dot shows the maximum cost distance equivalent, and the orange dot indicates the adjusted end point.

In each year reproduction was modeled by creating a list of all males within a given radius (1000 m for the simulations in this report) that could reach each female through a least-cost path movement (as described above). The number of eggs per female in each year was drawn from a Poisson distribution (characterizes discrete events with a low probability of occurrence) with lambda = 6 (parameter of the Poisson distribution similar to the mean), as this approximates the average number of eggs laid per year by Mojave desert tortoises (Mitchell et al. 2021). Since desert tortoises are known to have multiple paternity, each male within the mating radius had the ability to be the father to one or more eggs, and was selected at random (with replacement) for each egg. The genetic makeup of the offspring was assigned randomly (one allele at each locus drawn from the mother and the father) for each of 20 alleles. Offspring were produced with an equal sex ratio (U.S. Fish and Wildlife Service 2011) and their initial spatial locations were set as the location of the mother. The mother and father were recorded along with the local habitat value and zone (areas within landscapes predetermined by major roads and the railway). Each offspring was then assigned an age of 0 and a start year.

After movement and mating, tortoises were subject to mortality. For each tortoise a mortality risk score was calculated that considered individual age, habitat suitability, and the local tortoise density. Elevated risk relative to age was assessed for juveniles < 10 years old, as these tortoises were assumed to be approaching 100 mm in size (Medica et al. 2012) and at a higher risk of predation and other factors (e.g. susceptibility to climate extremes, predation, and dietary deficiencies, Segura et al. 2020). The partial risk score for elevated risk juveniles was calculated using a sigmoidal function (e.g. https://peerj.com/articles/4251/) where baseline annual morality for juveniles (15% per year) was increased for very young animals, reducing to baseline for mid aged animals. Older tortoises were also considered to have higher mortality risk (Medica et al. 2012). Starting at age 60 we increased mortality risk above the baseline adult mortality (6%, Figure 9.) The inverse of habitat suitability was used in calculations of mortality risk, such that low quality habitat (1) had a high risk, while higher quality habitat (0) had no added risk. Finally, localized density was calculated for each year using a Poisson point process density estimator of all tortoise locations with a bandwidth of 1000 m using the *density* function within the *spatstat* package (v.2.30, Baddeley and Turner 2005). The localized density per cell was calculated and an increased mortality risk was assigned to cells exceeding a density of 60 tortoises/km^2 using a sigmoidal function where risk at densities ≥ 70 tortoises/km^2 was assumed to be highest, and with mortality starting to increase at densities above 40 tortoises/km^2 (Figure 10). These numbers were selected based on simulation model thresholds that resulted in stable simulations, and field observations where these densities were seldom exceeded (Turner et al. 1984), especially during more recent surveys (Mitchell et al. 2021). The three partial risk scores (age, habitat, and density based) were then summed to create the total additional mortality risk for each individual. For each tortoise in each year the mortality was determined using a random Poisson draw using the risk score as lambda, and a random uniform number draw between 0 and 100. If the random uniform number was less than the risk score the tortoise was considered to have died during this year. For example, if a tortoise had an additive risk score of 10%, and the random number drawn was any of 1:10, the tortoise was considered to have died, while any number drawn from 11:100 resulted in the tortoise surviving for another year.

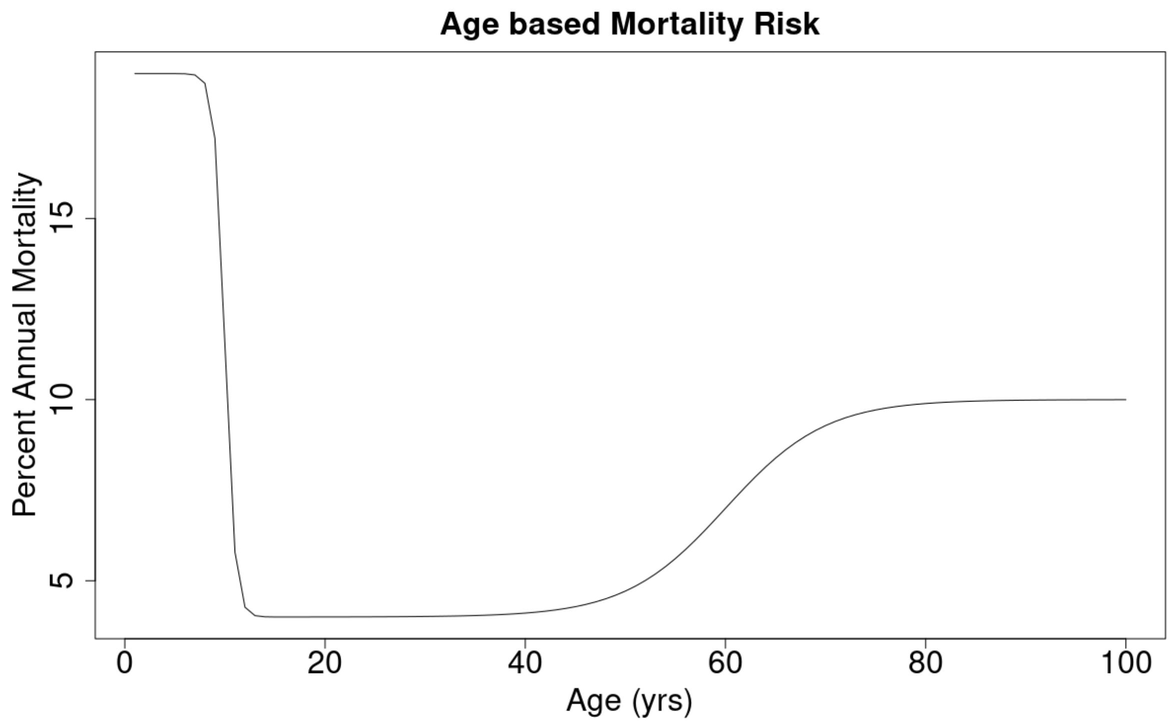


Figure 9 - Function used to assess desert tortoise mortality risk based on the age of the individual.

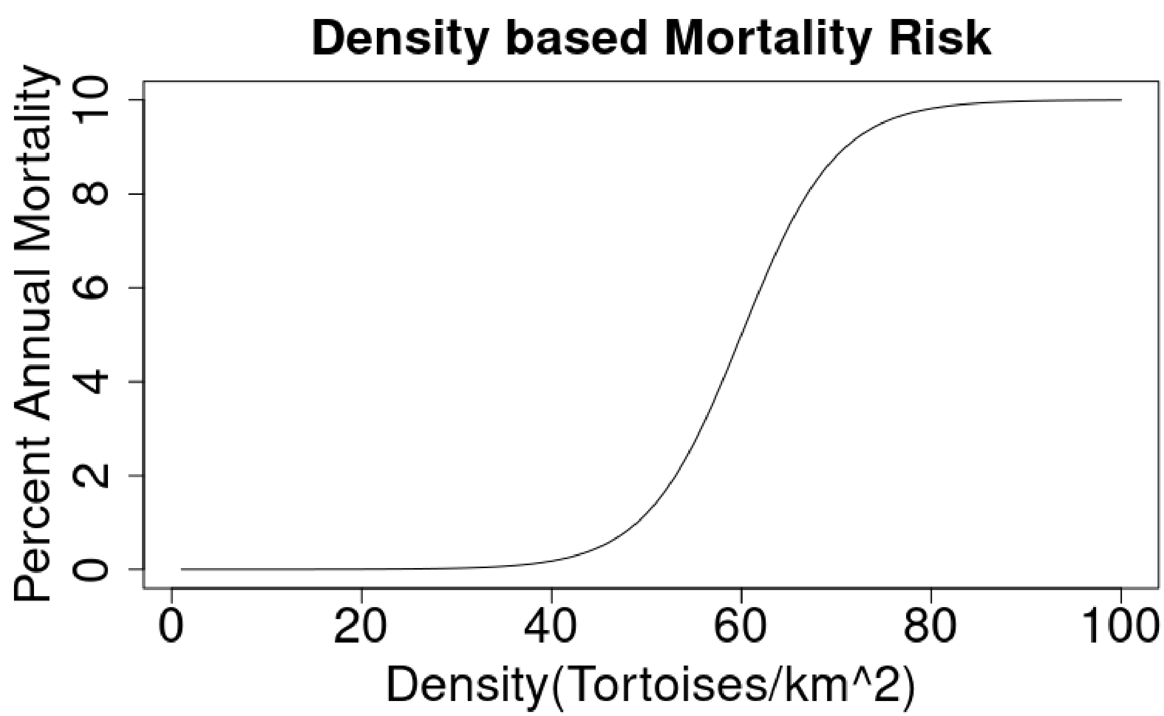


Figure 10 - Density based desert tortoise mortality risk used for simulations.

Simulation scenarios were evaluated for each of the study areas:

1. No Barrier – landscape with no anthropogenic disturbance based only on the habitat model/cost was run to create a 100-year baseline for an unimpeded landscape
2. Open Culverts – roadways and railways were considered barriers relative to traffic loads with all culverts assigned a value of 80% passable
3. Culvert 1 – roadways and railways were considered barriers relative to traffic loads with culverts assigned values from 0% to 80 % passable (provided by Clark County, Nevada), as described above. Current and predicted urbanization and solar development were included
4. Culvert 2 – roadways, railways, and culvert values followed the Culvert 1 scenario, except in the Ivanpah Valley, where culverts along a section of I-15 were given values of 80% passable, as described above. Current and predicted urbanization and solar development were included
5. Culvert 3 – roadways and railways followed the Culvert 1 scenario, but culverts were ranked based on their current state (e.g. many are not tied into tortoise fencing, and cannot be used by tortoises, despite otherwise sufficient condition). Current and predicted urbanization and solar development were included
6. Simple Barrier – roadways and railways were considered to be barriers to movement with all available culverts closed; however unintended barrier crossovers were infrequent, but possible. Additional urbanization was not included. This scenario represents the extreme of habitat fragmentation through absolute barriers on the landscape, without the confounding influence of habitat loss

Population demographics

In order to assess the results of the simulations, we examined demographic metrics like population size, mortality rate, reproduction rate, and annual displacement distance. By comparing metrics across scenarios, we can infer the consequences of landscape configuration on tortoise populations.

As connectivity is a key focus of this project, we also calculated metrics related to movement of tortoises between zones. Due to the nature of the simulation, there are both explicit and implicit movements between populations. Explicit movements are defined as a movement of a tortoise from one zone to another. However, movement can also occur implicitly – females are allowed to mate with males so long as they are within 1000 m and are not separated by a barrier, which means that tortoises in two different zones can mate so long as they are reachable. While this does not register as an explicit movement, it clearly implies the movement of a tortoise from one zone to another. Therefore, to analyze the movement between zones we looked at both the explicit and implicit movements.

For explicit movement, we kept track of the movement of tortoises between zones and then used this to analyze the number of immigrants and emigrants for each zone in each year. We also used this information to keep track of the pairwise movement between the zones, that is, the number of tortoises that moved between a pair of zones (regardless of direction). To identify implicit movements, we tracked the number of tortoises born each year whose parents were in different zones. For each pair of zones, we kept track of the number of hatchling tortoises with parents from those two zones. Analyzing movement metrics across simulations can indicate whether some scenarios allow more movement than others. In addition, by examining these yearly values over time for a single simulation, we can identify changes in connectivity over time, which is particularly useful for examining how future development may impact connectivity.

Population genetic structure and diversity

For each landscape samples were selected from standardized zones to best isolate key areas where anthropogenic disturbance might jeopardize connectivity and test which landscape scenario may offer improved gene flow. Samples (n = 1000) were randomly selected without replacement within years from forward-in-time simulation output files for genetic analyses. Sampling individuals across a zone, rather than in clumps, reduces incorrect interpretation in the presence of IBD (Schwartz and McKelvey 2008). In each scenario samples were taken by age class: old (≥ 17 years) or young (< 17 years) to evaluate differences in genetic signal.

Exact tests for HWE using a Monte Carlo procedure with 999 permutations were performed per locus in simulation years 1 and 100 for all individuals in each landscape using the package *pegas* v.0.11 (Paradis 2010) in R. The underlying assumptions of HWE include discrete generations in an infinite population with random mating and no migration, mutation, or selection (Waples 2014). Related individuals and large sample sizes (ex. highly variable loci or number of individuals) increase the likelihood of deviations (Hedrick 1999; Robertson and Hill 1984).

Because our simulations violated assumptions (overlapping generations, a finite population), as do many naturally occurring populations, not all individuals were unrelated, and we had large sample sizes both in terms of number of loci and number of samples; therefore, deviations were likely.

Spatial genetic analyses incorporate geographic information to detect discontinuities and infer population clusters based on patterns of genetic structure (Jombart et al. 2008). We tested for IBD between matrices of genetic and geographic distances with a Mantel test at year 100 (Mantel 1967). The observed genetic structure was compared to the distribution of random expectations using 999 Monte-Carlo permutations. Because both IBD and highly differentiated populations will result in significant differences, we used two-dimensional kernel density estimation plots to disentangle whether outcomes stemmed from a continuous (IBD) or discontinuous (clustered) population in *adegenet* v.2.1.5 (Jombart 2008). More complex and cryptic spatial patterns can be evaluated using multivariate methods, which are advantageous because they do not rely on HWE (Evanno et al. 2005; Jombart et al. 2008; Schwartz and McKelvey 2008). We used spatial principal component analysis (sPCA), a multivariate method that maximizes genetic variance in individual allele frequencies, accounts for spatial structure using a connection network, and allows for the presence of IBD (Jombart 2008). A relative neighbor's connection network with jittered geographic coordinates (redundant coordinates were not allowed) and Moran's I were used to detect spatial structure with 999 permutations (Moran 1948).

The potential genetic divergence resulting from each landscape scenario was calculated from simulation output genotypes. Divergence can be evaluated using F-statistics, which may be influenced by functional connectivity across the landscape. We evaluated pairwise genetic differentiation (F_{ST}) between zones at year 100 (Jombart 2008). Significance testing (999 permutations) for pairwise F_{ST} was calculated using *hierfstat* v.0.5-9 (Goudet 2005; Weir and Cockerham 1984). Genetic differentiation was predicted at each time step using simulated years 1 to 100 and tested for significant differences between zones using ANOVA in *rstatix* v.0.7.0 (Kassambara 2013; Nei 1973). Significance testing (paired *t*-tests) was conducted between years 1 and 100 to evaluate differences through time (Jombart 2008). Paired *t*-tests were also used to estimate differences between old and young animals through time.

Predicted genetic diversity (the extent of genetic variation within a group) statistics were calculated from simulation output genotypes of each landscape scenario by zone. Genetic diversity can be measured by allelic richness (*Ar*) and heterozygosity. Allelic richness expresses mean variation in nucleotide sequences at the same location standardized to sample size. Heterozygosity measures the proportion of loci with different alleles, and is reported as observed (heterozygotes at a locus divided by individuals sampled) and expected (calculated using allele frequencies as number estimated under HWE). Because observed heterozygosity (H_o) should align with expected values in randomly mating populations and our sample sizes were large we reported mean H_o values for each landscape scenario by selected zones. We tested for significant differences between observed and expected heterozygosity using paired *t*-test (Jombart 2008). Genetic diversity was predicted at each time step using simulated years 1 to 100 and tested for significant differences between zones using ANOVA in *rstatix* v.0.7.0 (Goudet 2005; Kassambara 2013). Significance testing (paired *t*-tests) was conducted between years 1 and 100 to evaluate changes through time. Paired *t*-tests were also used to estimate differences between old and young animals through time (Jombart 2008).

Results

We examined population demographics, genetic structure, and genetic diversity over a 100-year period at six landscape locations across Clark County, Nevada: Ivanpah Valley (IV), Lake Mead area (LM), North area (NO), Northwest corridor (NW), Pahrump area (PA), and Piute-Eldorado Valley (PV; Figure 4). Each of the six landscapes was modeled independently and the results are presented below. For more detailed genetic results, see the Supplemental Genetics Appendix.

Landscape: Ivanpah Valley

The Ivanpah Valley, situated on the Nevada-California border southwest of Las Vegas, Nevada, is an important corridor for desert tortoise connectivity that is under increasing pressure of development for solar energy and urban infrastructure associated with the Las Vegas metropolitan area. The valley is currently impacted by mining, roads, interstate highways, a railroad, urban development, OHV use, transmission line rights of way, and utility scale solar facilities (photovoltaic and solar thermal). Our urban growth modeling predicts increased development in the region associated with the construction of the new Jean Airport ca. 2040, with increased development thereafter (Figure 11).

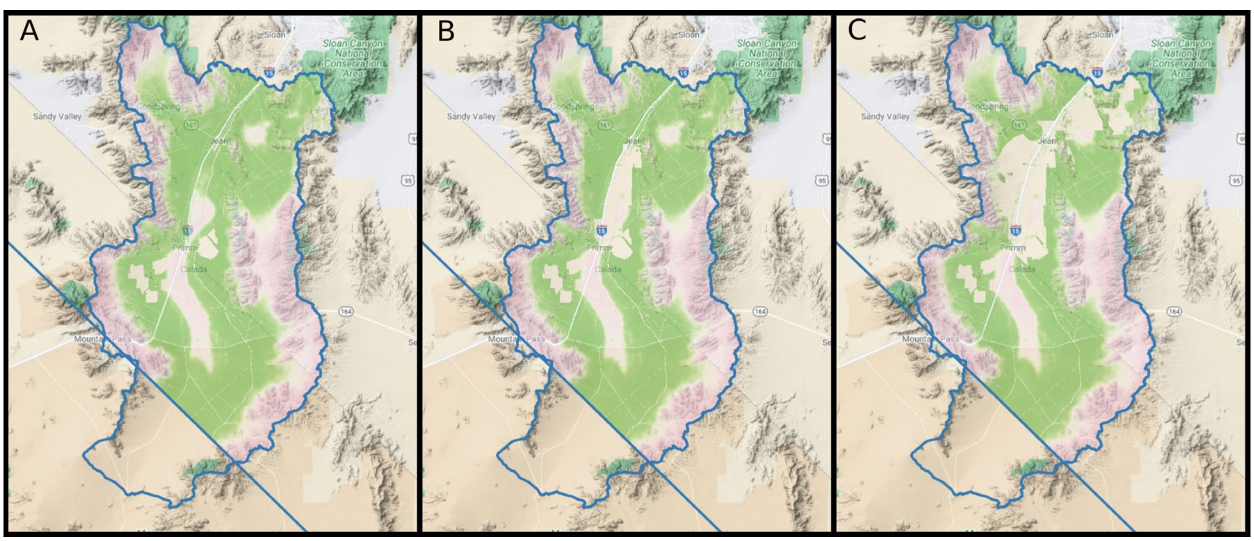


Figure 11 - The Ivanpah Valley study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020, B) 2050, and C) 2100.

The Ivanpah region was divided into six primary zones for analysis (Figure 12 and Table 1). These depict areas that are separated by key barriers i.e. I-15, and the railway, as well as smaller roadways that bisect habitat (Goodsprings Rd, Nipton Rd, and Ivanpah Rd). By and large the zones that are heaviest hit by development are Dry Lake, Nipton North, and Goodsprings South. Snapshots of area, and habitat quality at the beginning, middle and end of the simulation showed reductions in effective area (as urban development is removed from the habitat area) and changes in the average habitat quality (Table 2). Areas were reduced by 13 to 20% in the three zones most impacted by urban growth across the simulation. Habitat costs decreased in three of the six zones, indicating that the habitat lost was on average lower in cost (i.e. had higher habitat value). This is likely due to the overlap of new development with tortoise habitat.

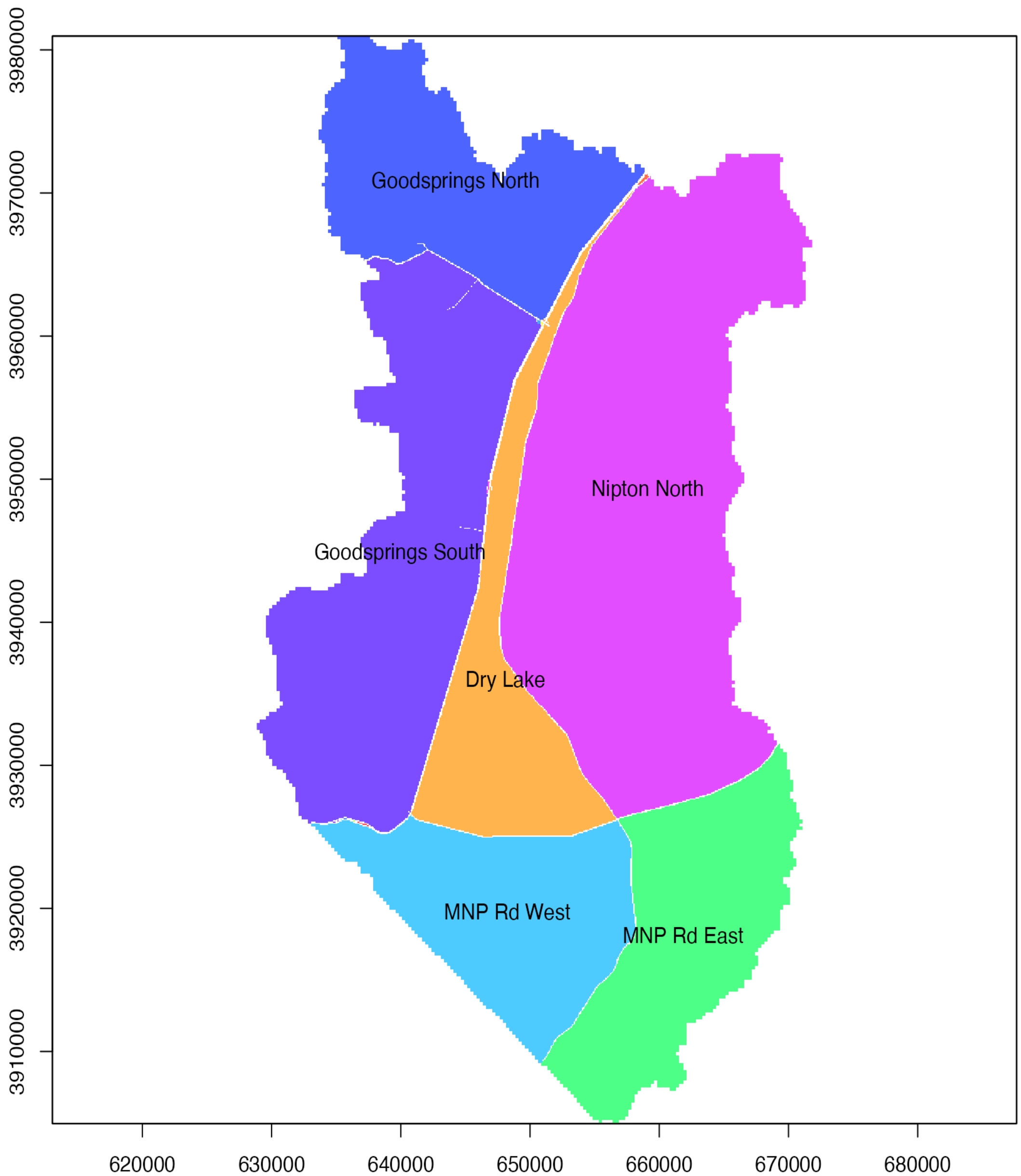


Figure 12 – Zones in the Ivanpah Valley study area that were used for analyses.

Table 1 - Ivanpah zones. Larger areas separated by prominent boundaries within the Ivanpah Valley area. (Note that MNP is being used as an abbreviation for Mojave National Preserve).

Zone	Zone Name	Description
8	Goodsprings North	West of I-15, north of Goodsprings Road
19	Nipton North	East of I-15, north of Nipton Road
28	Goodsprings South	West of I-15, south of Goodsprings Road
29	Dry Lake	East of I-15, north of Nipton Road, west of railroad
30	MNP Road West	East of I-15, south of Nipton Road, west of Ivanpah Road
31	MNP Road East	East of I-15, south of Nipton Road, east of Ivanpah Road

Table 2 - Ivanpah zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	Area (km^2)				Mean Cost			
	2020	**2050**	**2100**	**Loss**	**2020**	**2050**	**2100**	**Change**
Goodsprings North	254.44	254.44	247.70	6.74	0.39	0.39	0.40	0.01
Nipton North	677.73	659.12	587.22	90.50	0.30	0.30	0.33	0.03
Goodsprings South	402.35	398.55	332.21	70.14	0.33	0.33	0.38	0.06
Dry Lake	172.89	143.56	137.78	35.12	0.26	0.25	0.26	0.00
MNP Road West	224.15	224.15	224.15	0.00	0.18	0.18	0.18	0.00
MNP Road East	244.10	244.10	244.10	0.00	0.49	0.49	0.49	0.00

Demographics

The changes in area and in habitat resulted in changes in demographics over time. Adult death rates largely remained constant (although annually variable), except when major shifts in development occurred. For example, the largest urban growth occurred in the Nipton North, Goodsprings South, and Dry Lake zones, and these are realized in the Culvert 1, Culvert 2, and Open Culvert Scenarios. Increased mortality rates can be seen in Nipton North beginning in about year 40, Goodsprings South showed elevated rates after approximately year 50, and Dry Lake showed a large spike in mortality at year 25 with elevated mortality rates in the years following (Figure 13). Population levels over the same time frame show drops in the simulations that contain annual urban growth in the models (Culvert 1, Culvert 2, Culvert 3 and Open

Culverts; Figure 14). Dry Lake showed a sharp decline in population at year 24, with the initial build out of the Jean Airport, with slight improvements in the Open Culverts and Culverts 3 (Figure 14). Nipton North and Goodsprings South showed declines as the human population expanded in year 50 to 60, as these losses were associated with habitat growth. Population density grew slightly after the initial declines as tortoises reconfigured on the landscape, but recovery was never to the levels of the pre-build out population.

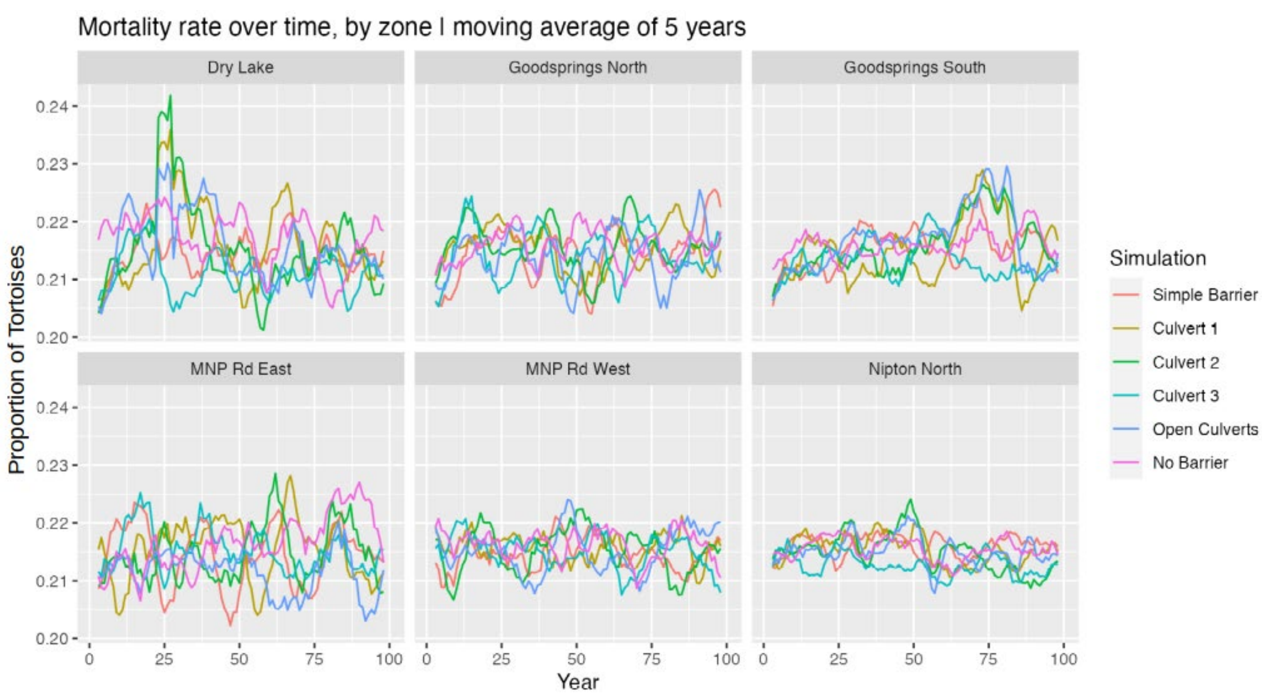


Figure 13 - Adult mortality rates in the Ivanpah Valley study area. Mortality proportions for adult tortoises are shown over time in each zone.

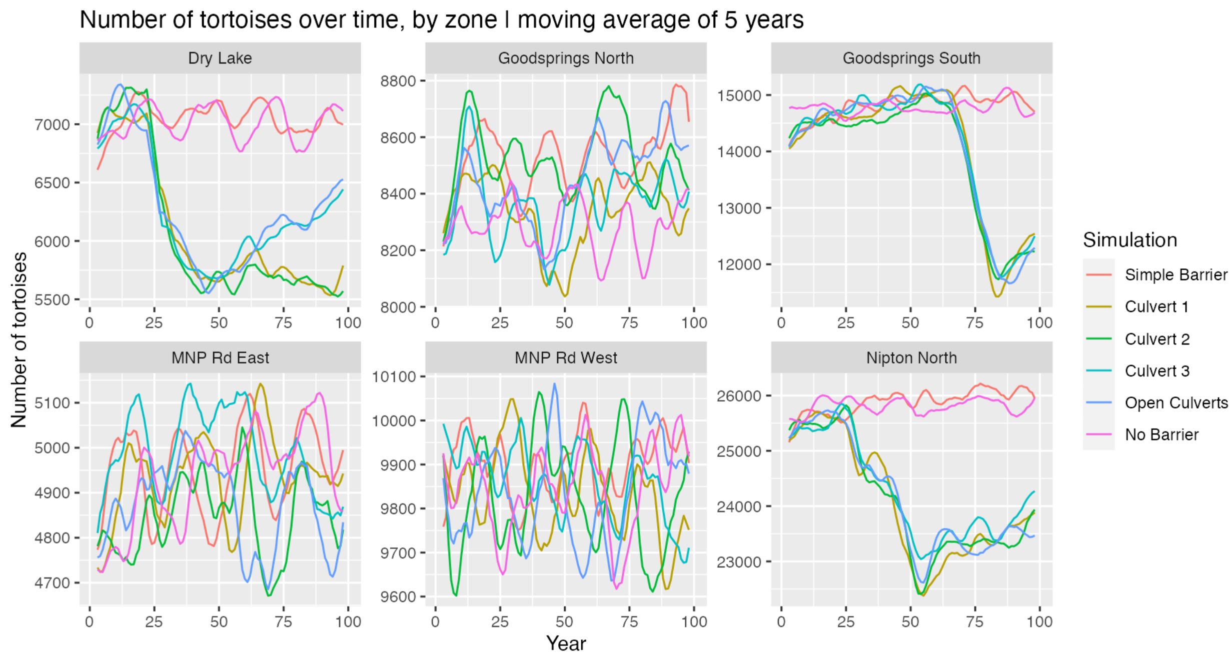


Figure 14 - Number of live tortoises in the Ivanpah Valley study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes.

Movements among zones can be illustrated in a variety of ways, and were generally similar among zones, but did differ in important ways. First there was a reduction of tortoises crossing zones by half or more when comparing a no barrier scenario to those with roads and culverts, and those with roads acting as barriers, which show almost no movement (Figure 15). The number of tortoises crossing zones in the Culvert 2 scenario appears similar to Culvert 1 and the Open Culverts scenario for Goodsprings North, and interestingly in Zones 30 and 31, although no changes to corridor passability were created in those areas between the Culvert 1 and Culvert 2 scenarios. The “current state” scenario (Culvert 3) showed a reduction in connectivity between zones 19 and 8 – which would occur near the Jean area, and while each of the culvert scenarios showed a decline, the current state had none (Figure 15). In contrast the Open culverts scenario had higher connectivity than any of the other culvert scenarios, indicating that connectivity could benefit from the improvement of all culverts. Each zone has a different configuration as to the potential for exchange of individuals due to both the barriers between them, but also the spatial arrangement of zones. A map of individuals that moved between adjacent zones, and how they differed under each scenario is shown in Figure 15. In general, and in all of the culvert based scenarios, there was substantial movement between zones on the eastern side of I-I5, which remained more of a barrier than minor roads or the railroad. This is likely due to the divided four lane configuration of the highway, where tortoises would need to move through two sets of culverts to reach the major zones on either side. Given that tortoise movements, in reality and as simulated, are not known to be overly large, this seems like a reasonable assessment of the nature of the barriers. The most connected zones were the Dry Lake to Nipton north, Goodsprings South to Goodsprings North, MNP Road East and West, MNP Road East to Nipton North, and MNP Road West to Dry lake. The No Barrier simulation highlights the amount of movement that is restricted due to the barriers in all of the simulations, which have emigration and immigration values much closer to the barrier simulation (Figure 16).

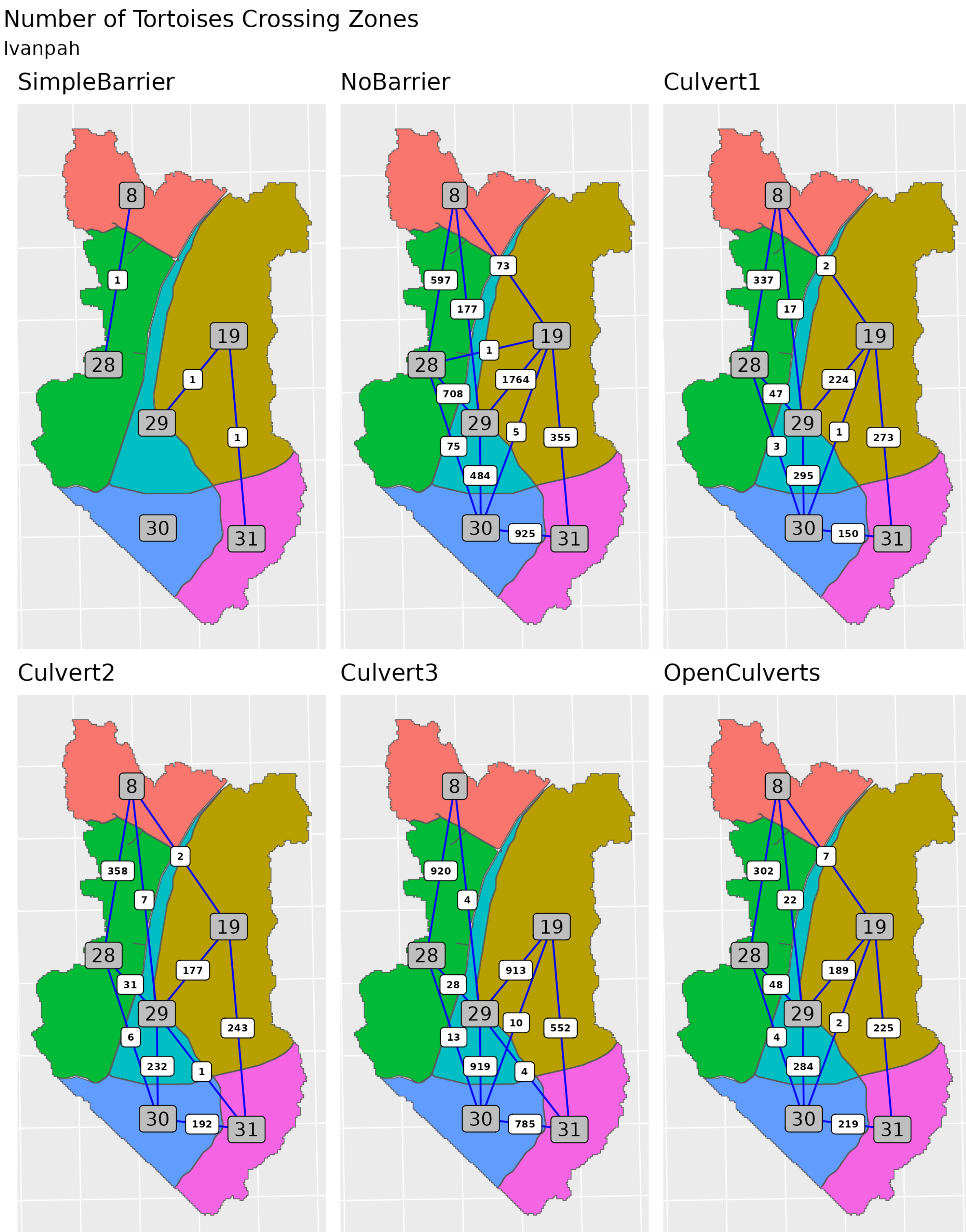


Figure 15 - The number of tortoises in the Ivanpah Valley study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels; zone names are given in Table 1.

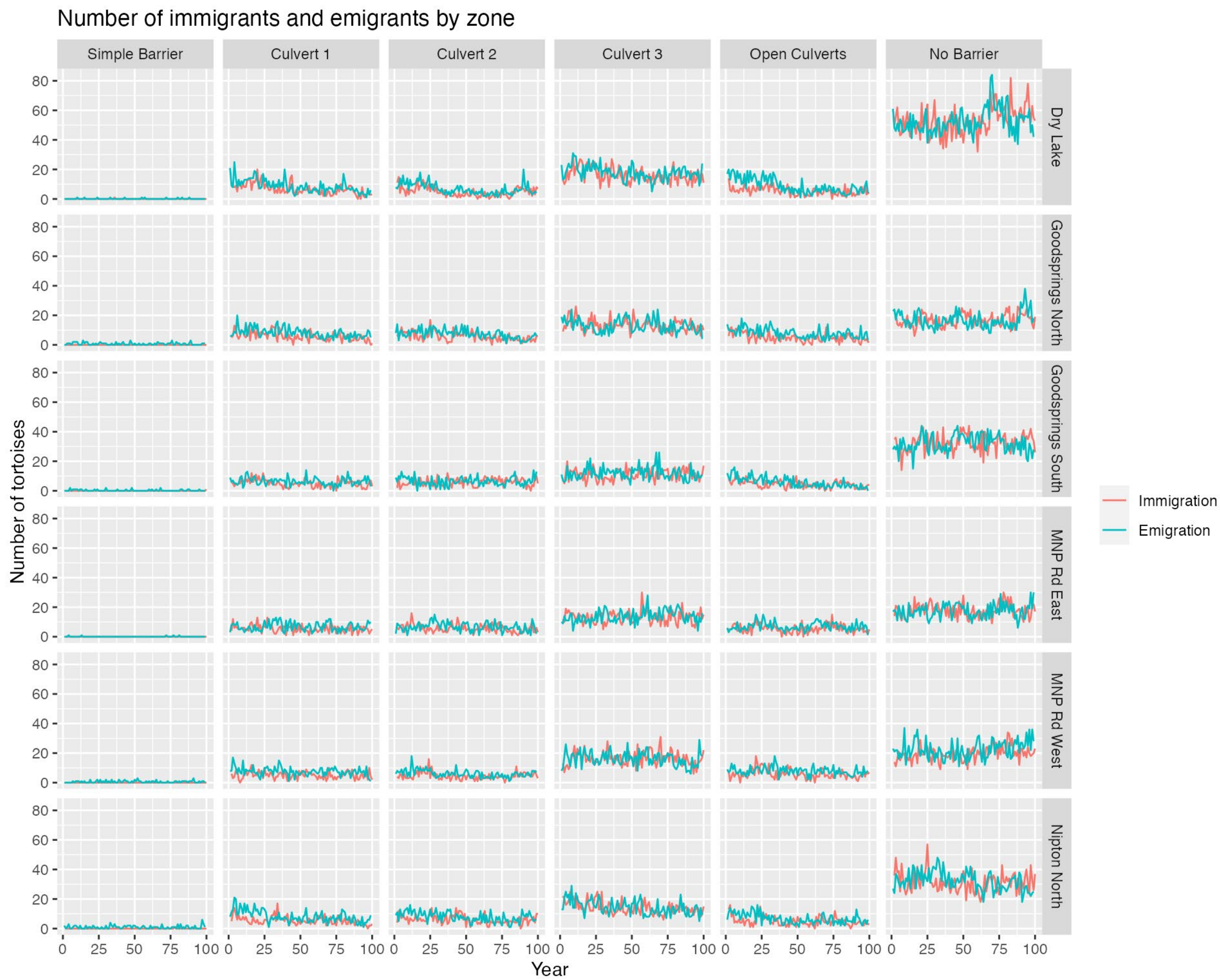


Figure 16 - Desert tortoise immigration and emigration in the Ivanpah Valley study area. Immigration and emigration are shown over time by zone for each scenario.

The number of tortoises mating was highest in the No Barrier simulation, and nonexistent in the Simple Barrier (Figure 17). For all culvert scenarios, connectivity was comparable and fell over time as a result of increased isolation among zones (Figure 17). Connectivity between zones was generally quite low; however, in pairwise comparisons between specific zones Culvert 1 and Culvert 2 appear to have maintained connectivity marginally better than Culvert 3 between Goodsprings North and Dry Lake; however, connectivity appears to be entirely lost in this area regardless of culvert scenario by year 30 as there are very few offspring from parents in different zones (Figure 18). This is likely due to current and predicted habitat loss from development in the area, as the only scenario that forecasted stable connectivity between these zones was No Barrier (Figure 18). Over time the predicted declines in connectivity throughout the Ivanpah Valley study area could result in further genetic isolation, but as this occurred 30 to 50 years into the simulations it is unlikely that the simulations were long enough to capture the full impact that would likely result from anthropogenic disturbance. The summary plot for this region showed population declines in simulations that included urbanization and solar (Figure 19).

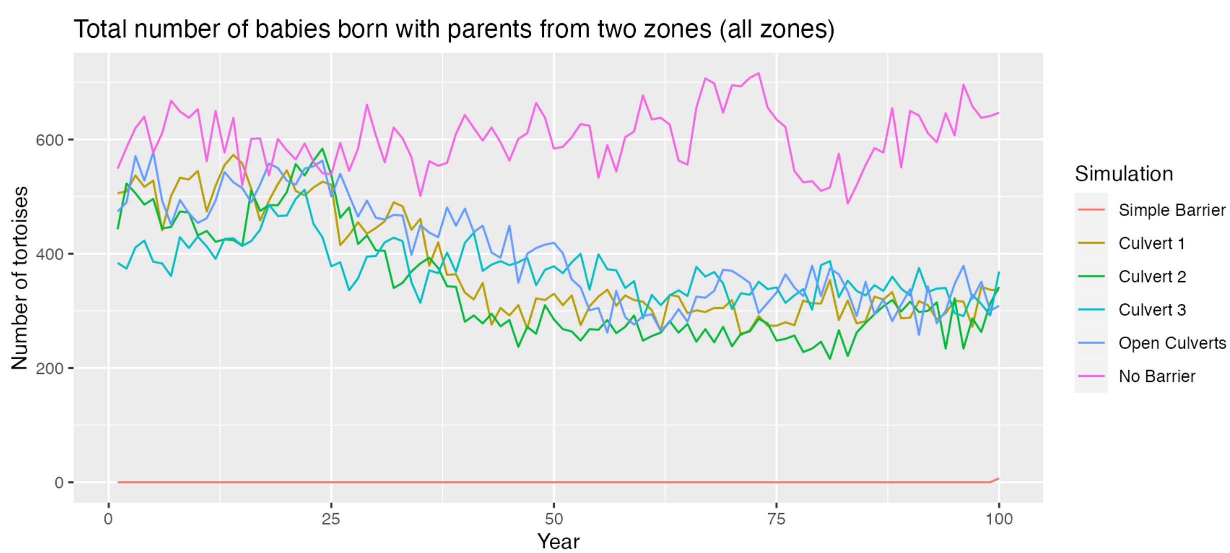
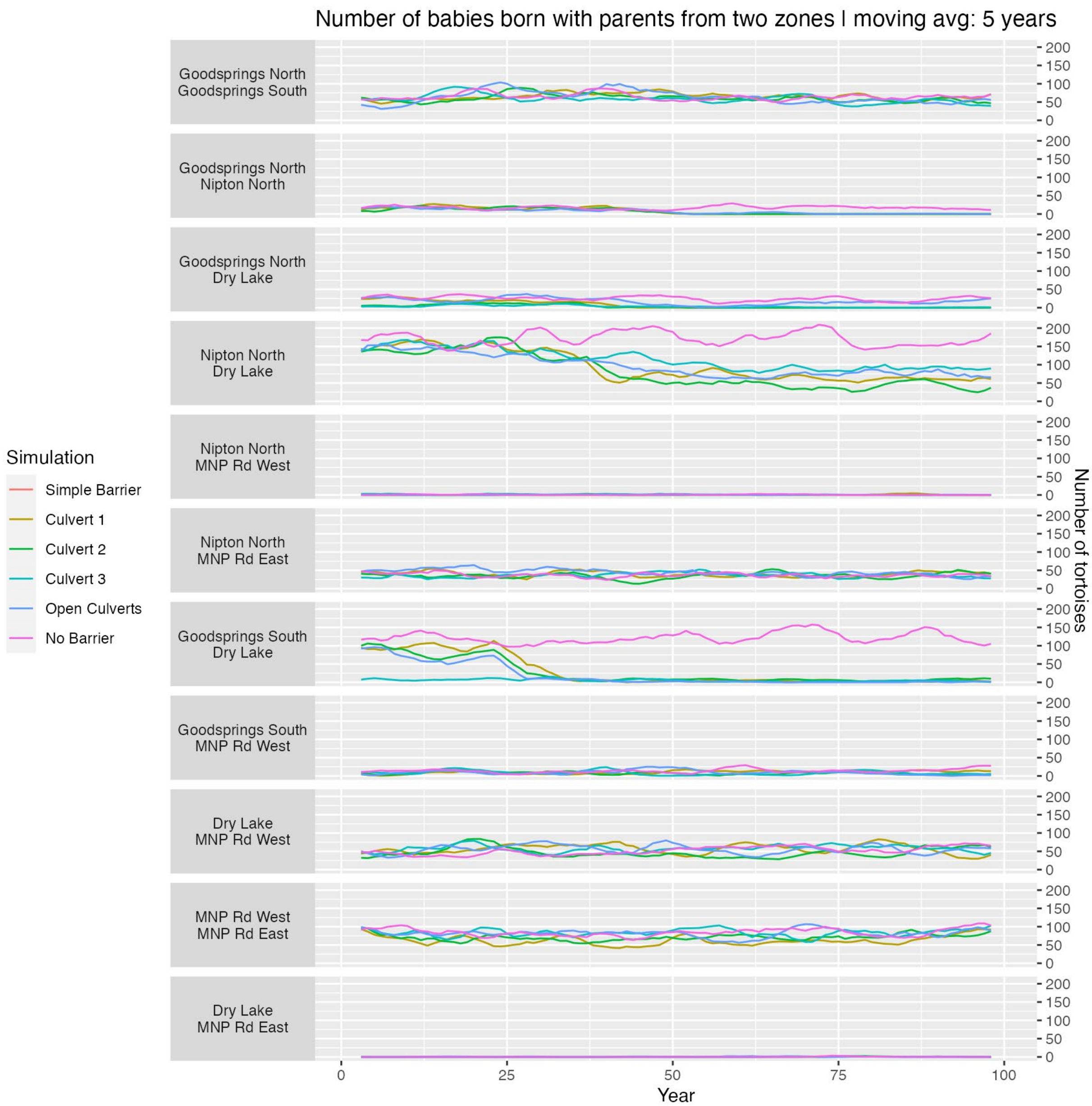


Figure 17 - Number of desert tortoises mating in the Ivanpah Valley study area. Mating is averaged across zones over time for each scenario.



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651 Figure 18 - Ivanpah Valley study area moving average of the number of offspring with parents
652 originating in adjacent zones. Average values are displayed over time by zone for each scenario.
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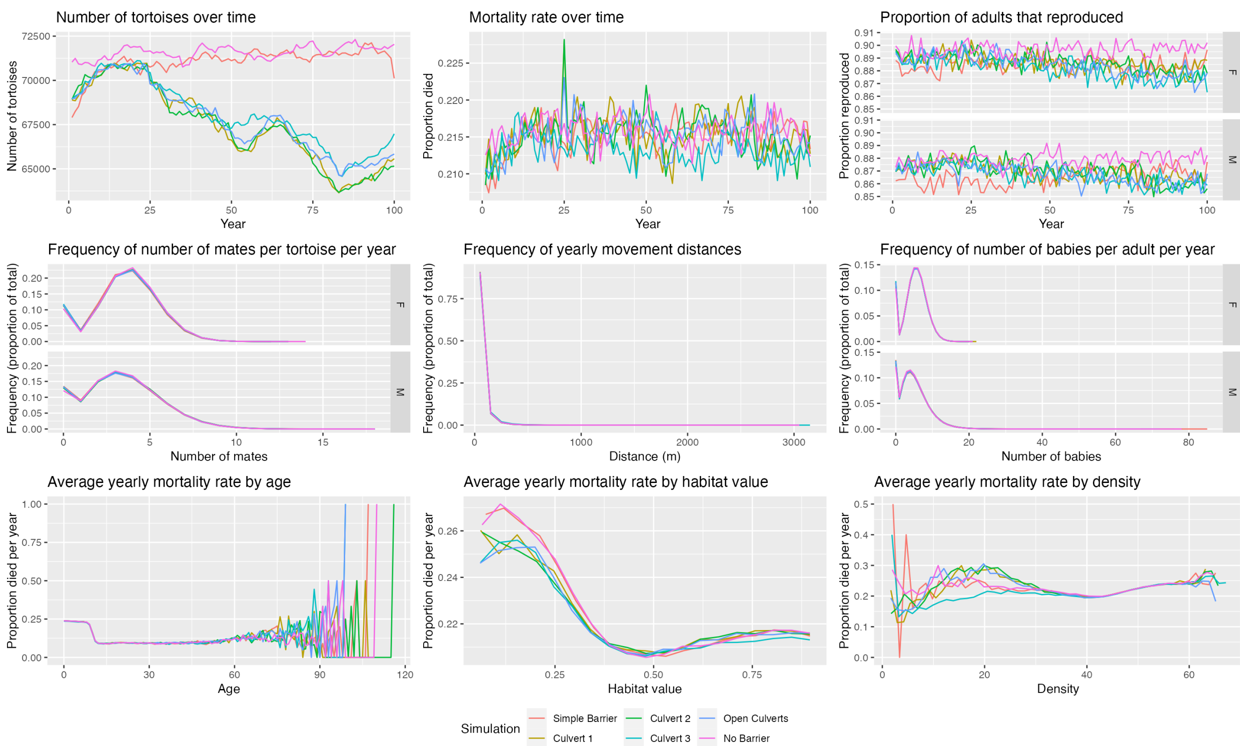


Figure 19 - Ivanpah Valley study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Of 20 microsatellite loci 17 to 19 were out of HWE by year 100; therefore, caution should be exercised when interpreting results where HWE is assumed. Genetic differentiation values between zones indicated that the Closed Culvert scenario did not allow connections (F_{ST} values < 0.05; see the Supplemental Genetics Appendix for F_{ST} tables). Additionally, F_{ST} increased significantly from year 1 to year 100 in all scenarios except the No Barrier (t-test *p*-value > 0.05). In the Culvert 1 scenario F_{ST} increased markedly in Dry Lake, while in the Open scenario it increased most in Goodsprings South, likely as the result of increased habitat loss and difficulty crossing linear features in these scenarios (Figure 20). Allelic richness tended to decrease more notably in Dry Lake with increased urbanization scenarios (Open, Culvert 1, and Culvert 2). Additionally, Goodsprings North was predicted to show marked decreases in the Culverts 2 scenario (Figure 21). Heterozygosity was similarly impacted, with the Open scenario indicating drops at Dry Lake and Goodsprings South and the Culvert 1 scenario showing marked declines at Dry Lake and Nipton North (Figure 22).

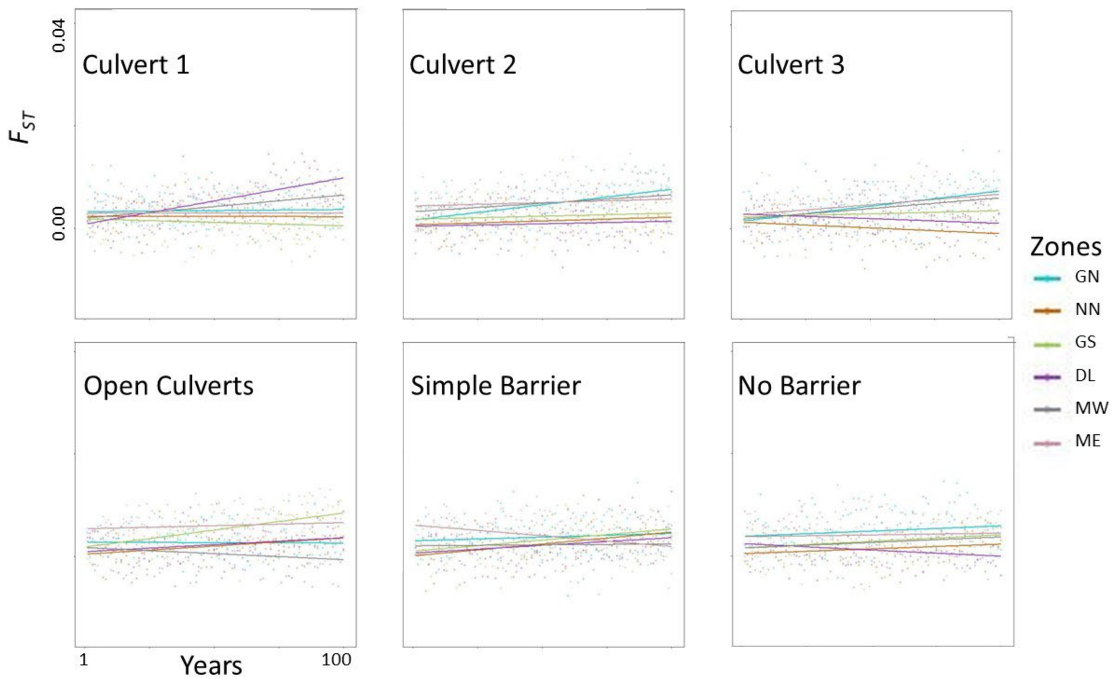


Figure 20 - Ivanpah Valley study area genetic differentiation (F_{ST}) over time by zone for each scenario. Zones are Goodsprings North (GN), Nipton North (NN), Goodsprings South (GS), Dry Lake (DL), MNP Road West (MW), and MNP Road East (ME).

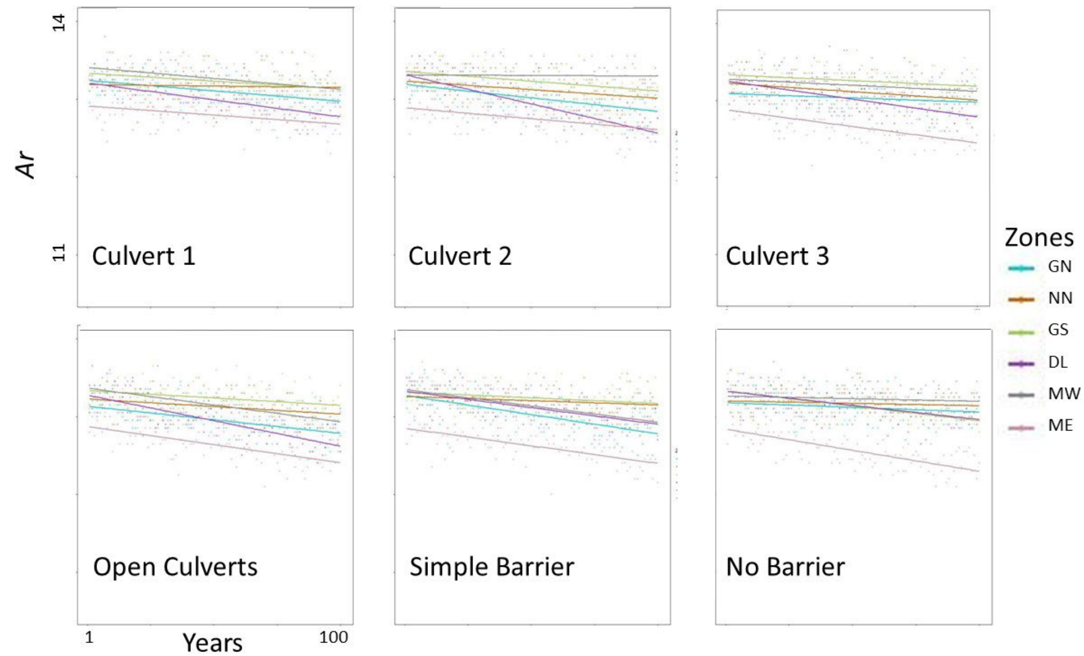


Figure 21 - Ivanpah Valley study area allelic richness (*Ar*) over time by zone for each scenario. Zones are Goodsprings North (GN), Nipton North (NN), Goodsprings South (GS), Dry Lake (DL), MNP Road West (MW), and MNP Road East (ME).

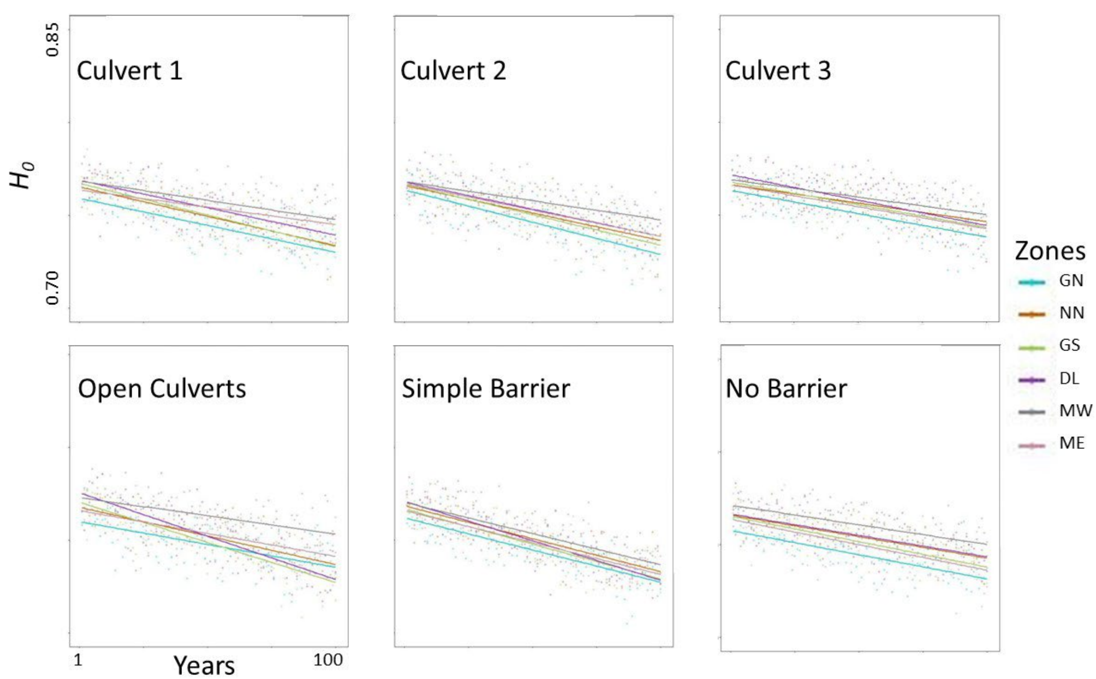


Figure 22 - Ivanpah Valley study area heterozygosity (H_o) over time by zone for each scenario. Zones are Goodsprings North (GN), Nipton North (NN), Goodsprings South (GS), Dry Lake (DL), MNP Road West (MW), and MNP Road East (ME).

Key takeaways from Ivanpah Valley simulations

Tortoises in all barrier simulations had similar demographics. Urban growth predicted a loss of individuals in the affected zones, but populations stabilized, albeit at lower population levels. While populations did appear stable after disturbance, it is important to note that populations in the smaller affected zones fell to levels of approximately 5000 to 6000 which is where minor changes in genetics started to become detectable with respect to Ar, F_{ST}, and H_o. In addition, mating of adults across barriers showed a reduction in time, as the effects of growth matured, and this could lead to further genetic isolation over time frames outside of the 100-year simulations presented here. Taken together, these results indicate that in the Ivanpah Valley habitat loss may have a greater impact on desert tortoises than linear features on the landscape, but that when populations in isolated zones become small enough genetic differentiation was detectable within the scope of 100 simulation years. For all culvert scenarios connectivity is lost over time due to habitat loss in the area. Because habitat loss has a large impact on this area and is predicted to increase, any efforts to offset or reduce planned or future development, or restrict it to already disturbed areas, in this area are highly recommended. Culvert scenario recommendations based on simulation results:

- The open Culvert scenario outperformed all others, indicating that there is the potential to increase connectivity as more culverts are opened – which can be seen in the increasing connectivity moving from more restrictive (Culvert 3) to less restrictive (Culvert 2, and then Culvert 1) to fully open scenarios (Open Culverts).
- The Open Culverts, Culvert 1, and Culvert 2 are similar; however, the Open Culverts or Culvert 2 scenario is predicted to best maintain connectivity along Goodsprings Road.

- All culvert scenarios performed similarly along secondary roads (e.g. Nipton Road and Goodsprings Road) with high connectivity.

Landscape: Lake Mead Area

The Lake Mead area is situated along the Nevada-Arizona border east of Las Vegas, Nevada along the Colorado River. The area is largely composed of a National Recreation Area, a recently designated National Monument, a State Park, and U.S. Bureau of Land Management lands. The area also contains a few small urban areas. Tortoise habitat is naturally fragmented in this region, with splits due to the Colorado, Muddy, and Virgin Rivers, as well as rugged terrain between areas of predicted higher habitat suitability. The Lake Mead area within Nevada is bisected by Northshore Road, which effectively runs from Henderson to Overton at the northern extent. Habitat within the area is largely protected and there is little expectation of urbanization or development. There is OHV use, and mining activity on U.S. Bureau of Land Management lands in the west, as well as the northern portion in and around Overton, Logandale, Mormon Mesa, and Goldbutte (Figure 23).

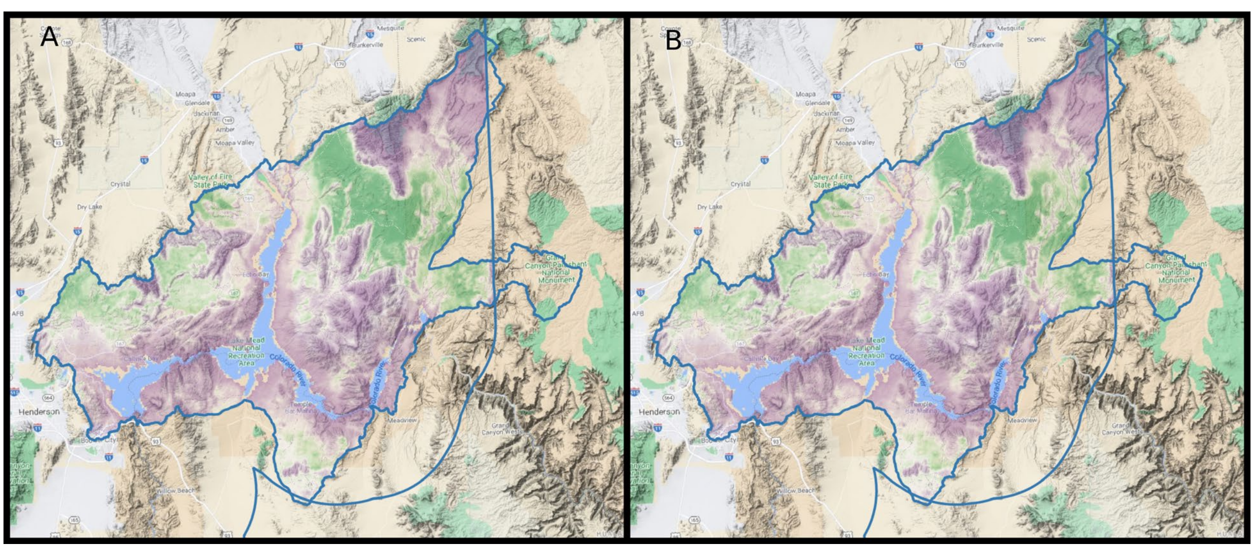


Figure 23 - The Lake Mead study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020 and B) 2100.

The region was divided into seven primary zones for analysis (Figure 24 and Table 3). These depict areas that are separated by key barriers in the region. The Colorado River separates the Arizona zones (Temple Bar and Fortification Hill) from the rest of the zones. The Northshore road separates the Northshore South and North zones, as well as the Lake LV East area. The Muddy and Virgin rivers isolate the St Thomas zone. Gold Butte, the largest zone, is isolated from the others by both the Colorado and Virgin Rivers. Snapshots of habitat area indicate little development with the exception of the loss of 10 km^2 within the Northshore North zone, representing approximately a 1% change in area. The minimal growth in our simulations did not change the predicted average habitat value of the area and all zones remained unchanged (Table 4).

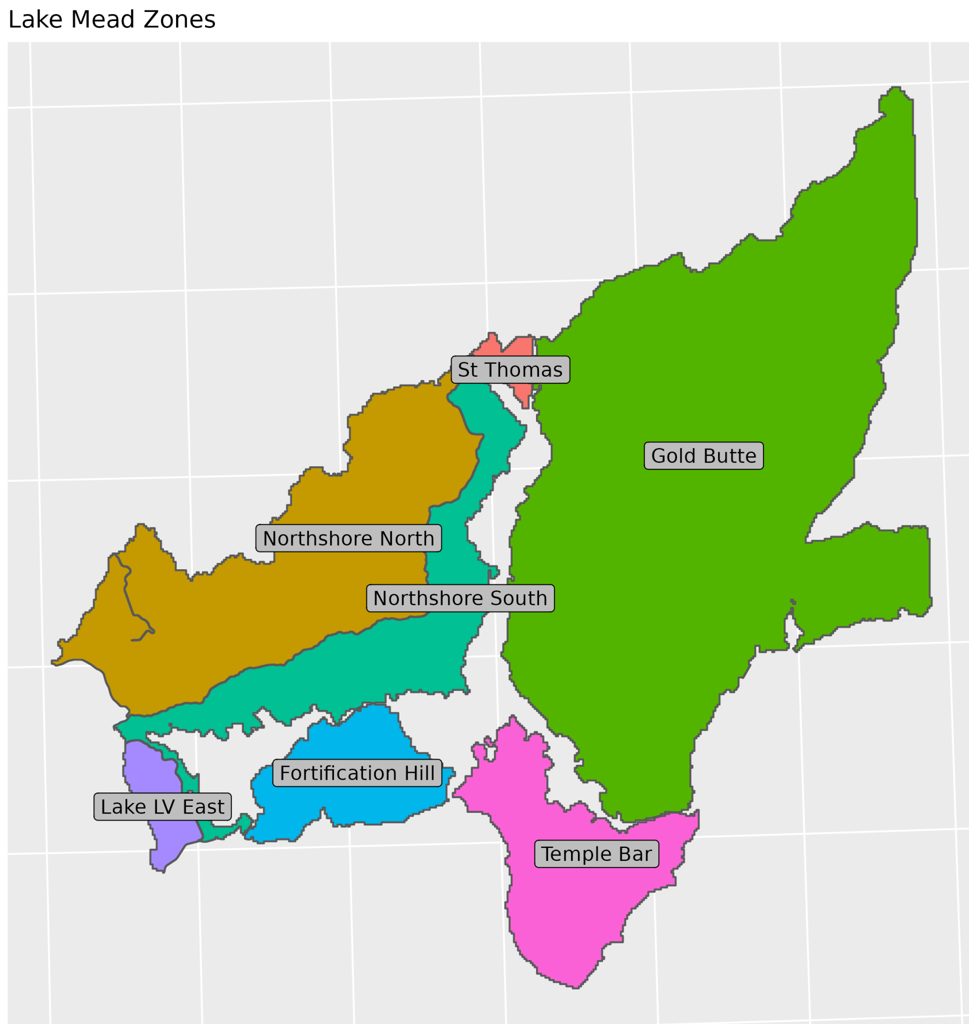


Figure 24 – Zones in the Lake Mead study area that were used in analyses.

Table 3 - Lake Mead zones. Larger areas separated by prominent boundaries within the Lake Mead area.

Zone	Zone Name	Description
10	Northshore North	North of Northshore Road
18	Northshore South	South of Northshore Road, east portion
21	Lake LV East	Lake Las Vegas east
3	St Thomas	Saint Thomas Gap
23	Temple Bar	Temple Bar
15	Gold Butte	Gold Butte
19	Fortification Hill	Fortification Hill

Table 4 - Lake Mead zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	**Area (km^2)**				**Mean Cost**			
	2020	**2050**	**2100**	**Loss**	**2020**	**2050**	**2100**	**Change**
Northshore North	857.57	857.57	847.40	10.17	0.47	0.47	0.47	0.00
Northshore South	460.01	460.01	459.96	0.05	0.73	0.73	0.73	0.00
Lake LV East	75.11	75.11	75.11	0.00	0.68	0.68	0.68	0.00
St Thomas	32.13	32.13	32.13	0.00	0.61	0.61	0.61	0.00
Temple Bar	408.12	408.12	408.12	0.00	0.63	0.63	0.63	0.00
Gold Butte	2464.56	2464.56	2464.56	0.00	0.56	0.56	0.56	0.00
Fortification Hill	242.80	242.80	242.80	0.00	0.77	0.77	0.77	0.00

Demographics

Population demographics over time were stable, but variable in the smaller zones with smaller tortoise populations. The zones with the largest fluctuations in mortality rates were Fortification Hill, Lake LV East, and St Thomas (Figure 25). Population levels showed increases over time in several zones across most scenarios, including Gold Butte, and Northshore North and South, while the others remained stable, but variable (Figure 26). Based on the number of tortoises, the Open Culverts scenario performed better than other culvert scenarios in Gold Butte and Lake LV East, while Culvert 1 appeared better in Fortification Hill and St. Thomas. Both Open Culverts and Culvert 1 functioned comparable in the Northshore South and Northshore North zones (Figure 26).

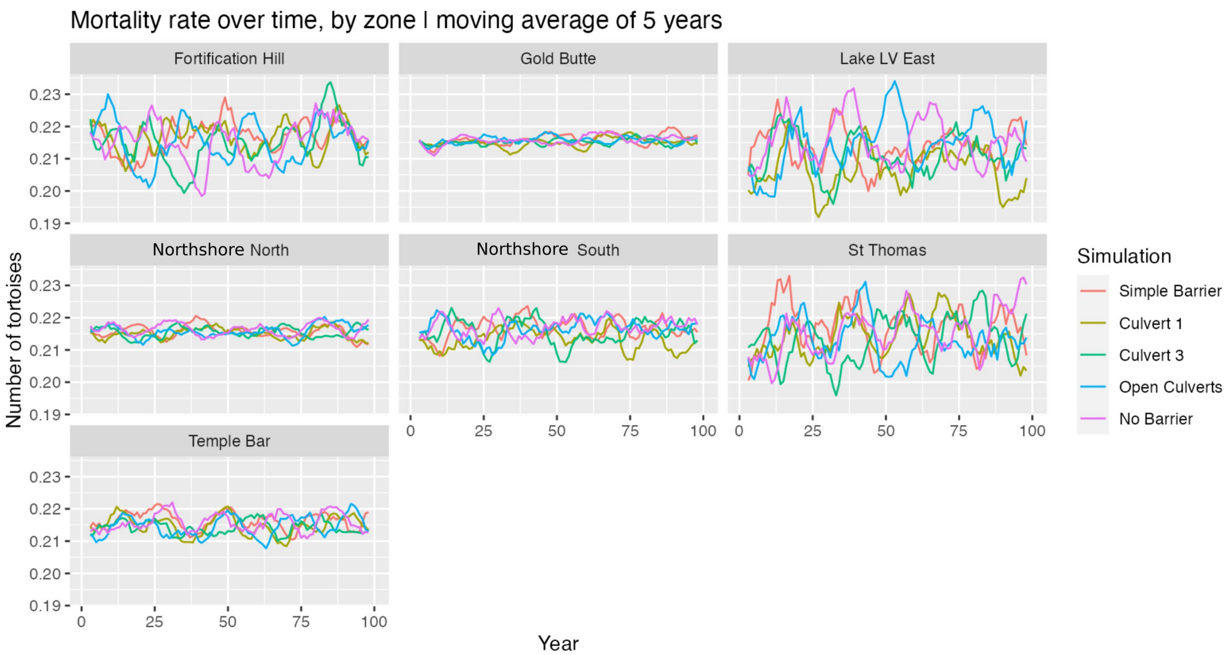


Figure 25 - Adult mortality rates in the Lake Mead study area. Mortality proportions for adult tortoises are shown over time in each zone.

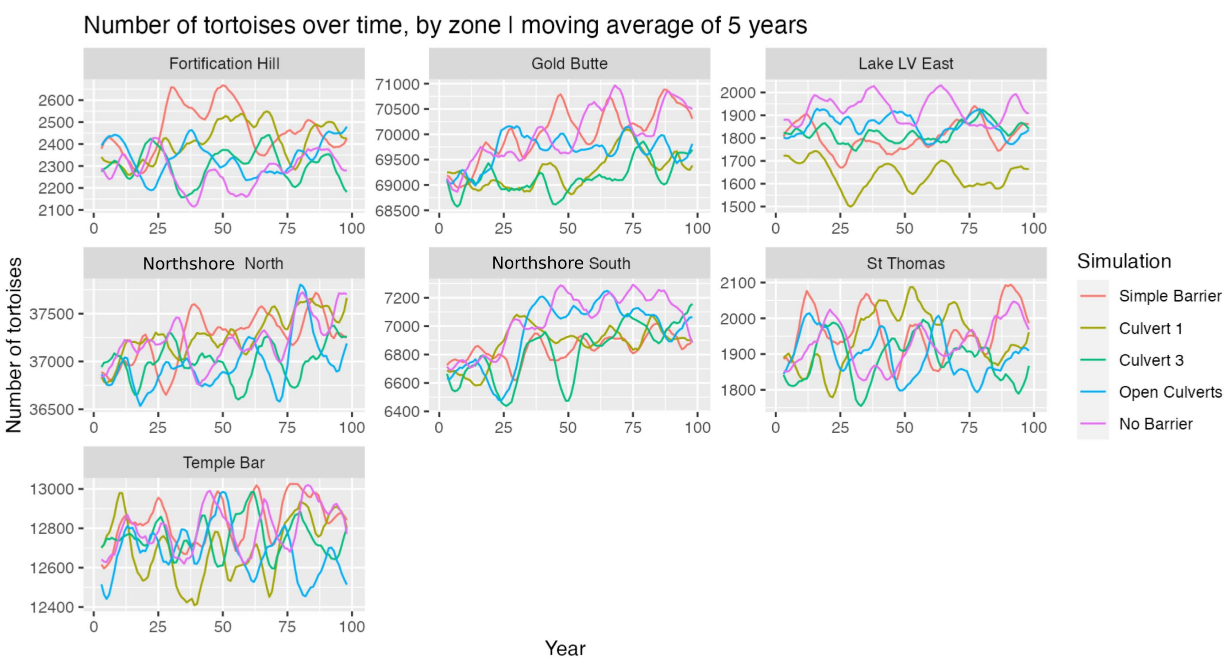


Figure 26 - Number of live tortoises in the Lake Mead study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes.

Movements among zones were limited in this study area, with the most movement occurring between Northshore North and South. There was an occasional movement detected between St Thomas and adjacent zones, but this was due to the narrow separation of the rasters depicting the rivers. Consequently, the zones that were monitored for demographics and genetics were generally isolated (Figure 27). The number of movements between zones relative to the culvert/growth scenarios was similar to that seen in other study areas. The Simple Barrier showed effectively no movement, the No Barrier scenario showed movements approximately 1/3 higher than the culvert scenarios, indicating that Open Culverts, Culvert 1, and Culvert 3 functioned comparably (Figure 27). Movements were constrained throughout the zones with the exception of Northshore North and South, which also showed the only substantial levels of immigration and emigration in all scenarios except the Simple Barrier (Figure 28).

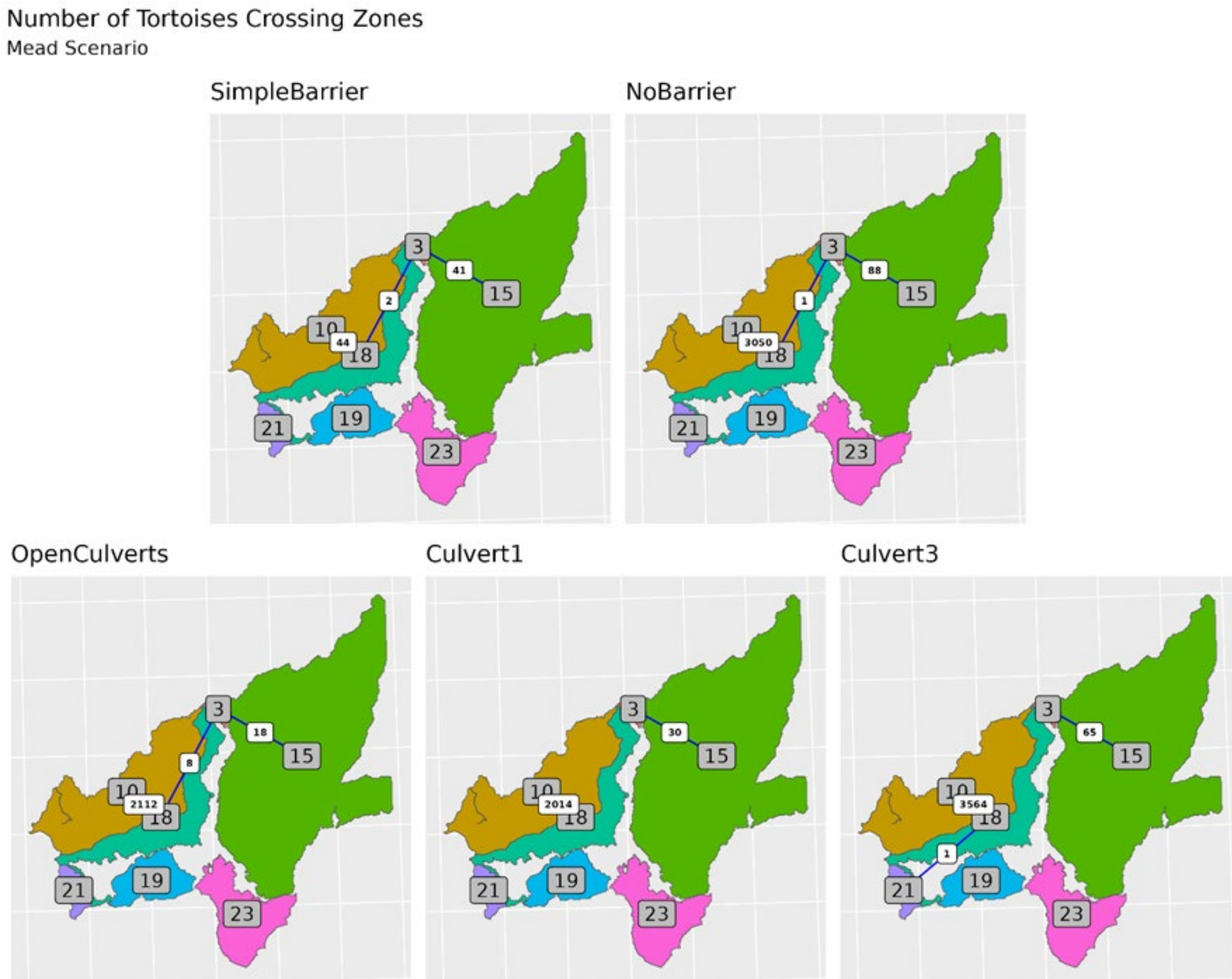


Figure 27 - The number of tortoises in the Lake Mead study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels, and zone names are given in Table 3.

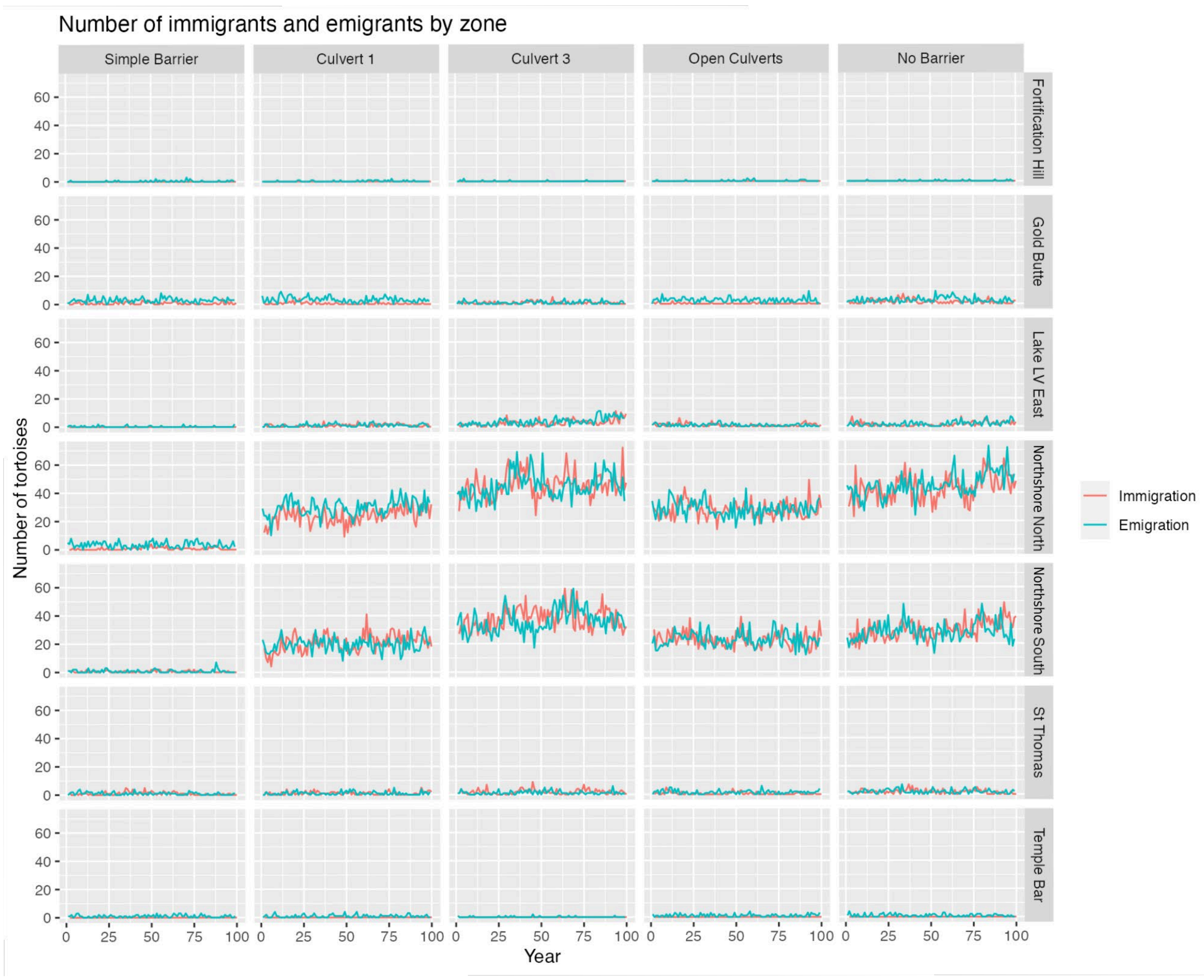


Figure 28 - Desert tortoise immigration and emigration in the Lake Mead study area. Immigration and emigration are shown over time by zone for each scenario.

Offspring produced by parents across zones remained relatively constant over time among all scenarios, except the Simple Barrier, which predicted connections near zero, as expected (Figure 29). Offspring produced by parents across zones was largely constrained to Northshore North and South, with all culvert scenarios comparable to the No Barrier simulation (Figure 30). There was minimal exchange between tortoises in the St Thomas zone and adjacent zones (Figure 30). The demographic summary for Lake Mead indicated similar overall metrics among all scenarios, although Culvert 3 tended to have marginally lower overall population numbers than Culvert 1 (Figure 31).

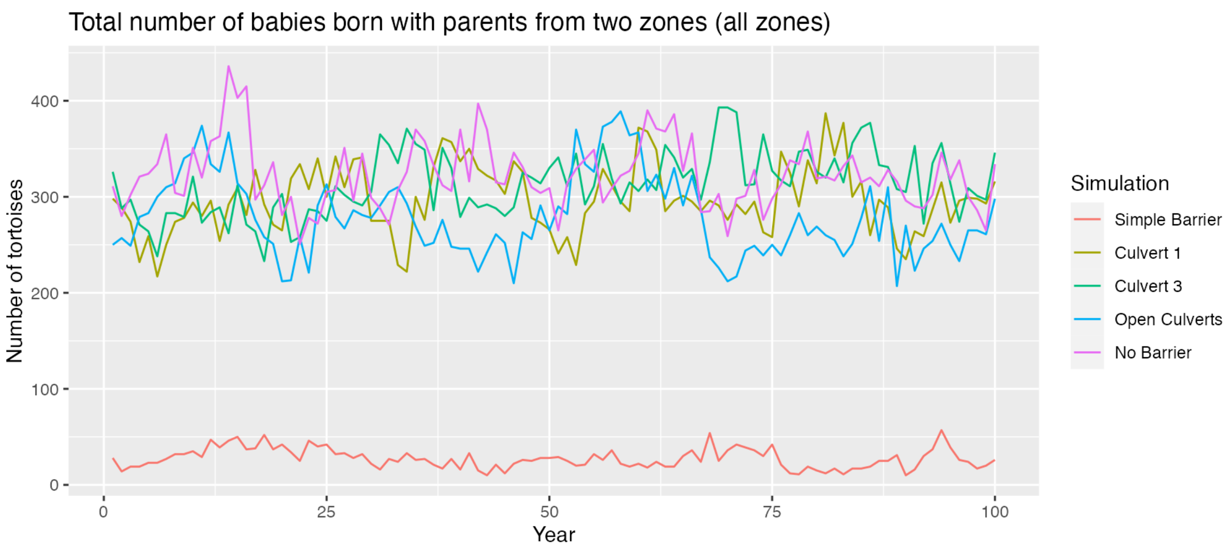


Figure 29 - Number of desert tortoises mating in the Lake Mead study area. Mating is averaged across zones over time for each scenario.

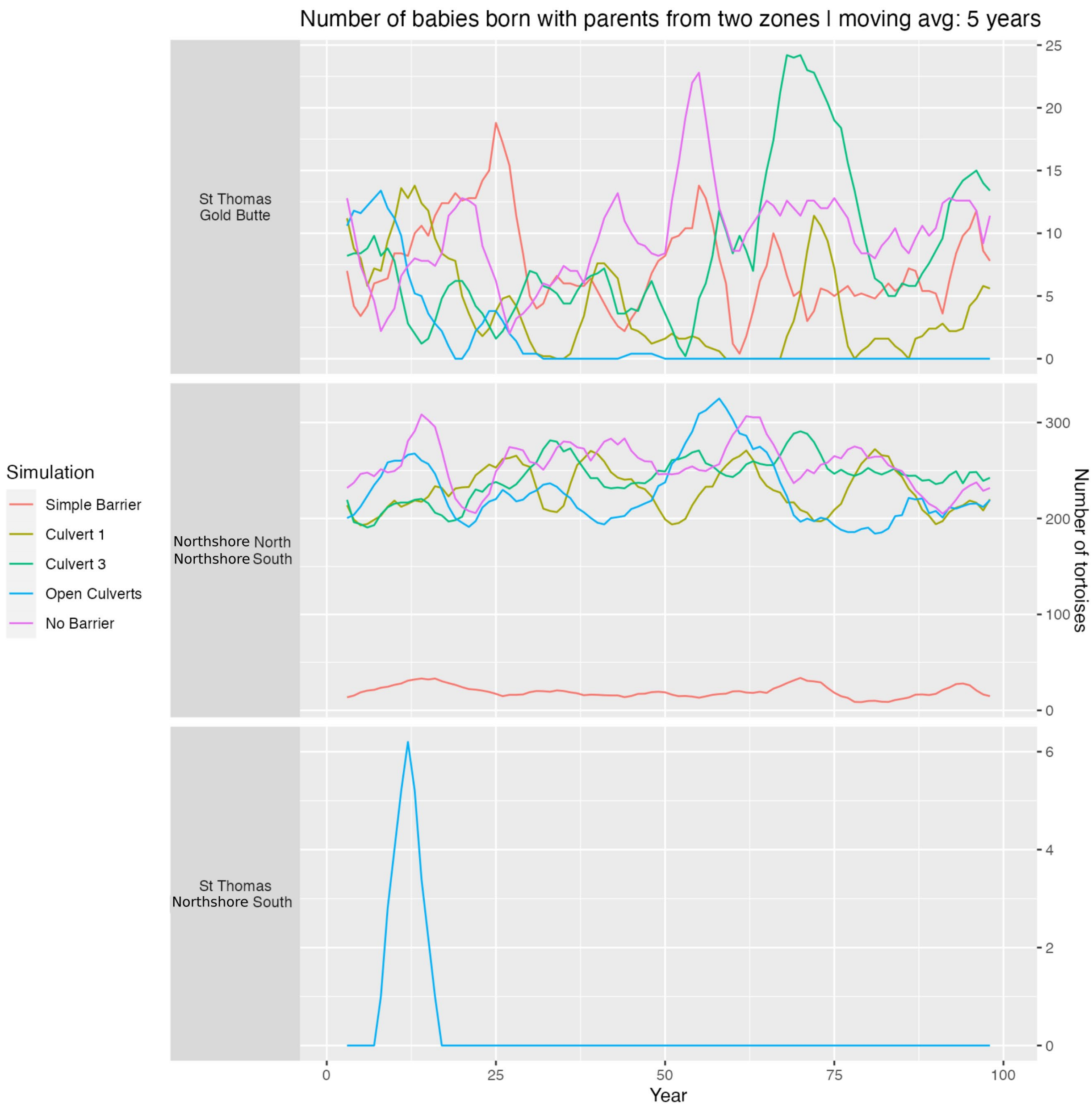


Figure 30 - Lake Mead study area moving average of the number of offspring with parents originating in adjacent zones. Average values are displayed over time by zone for each scenario.

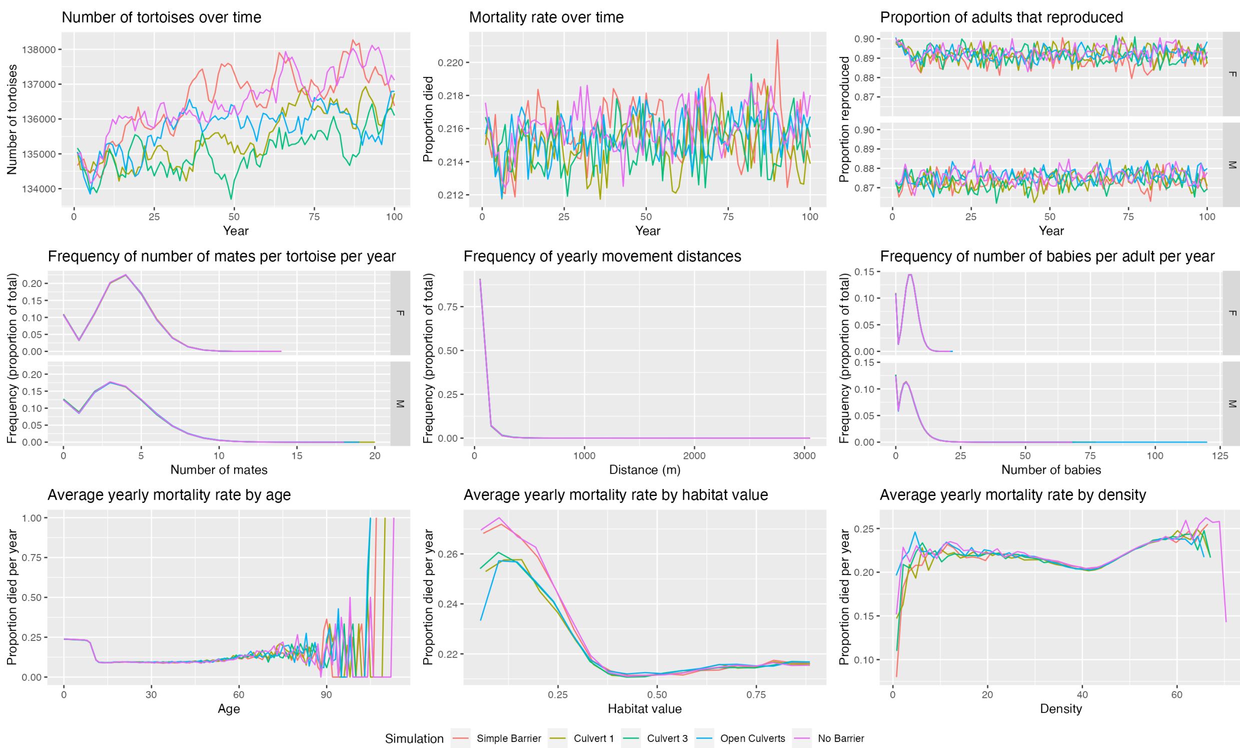


Figure 31 - Lake Mead study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Of 20 microsatellite loci all were out of HWE by year 100 in each scenario; therefore, caution should be exercised when interpreting results where HWE is assumed. Genetic metrics, by and large, did not reveal any telling differences between scenario. However, H_o was found to differ by zone in all barrier scenarios (Open Culverts, Culvert 1, Culvert 3, and Simple Barrier; *p*-value < 0.05), but not in the No Barrier (Figure 32).

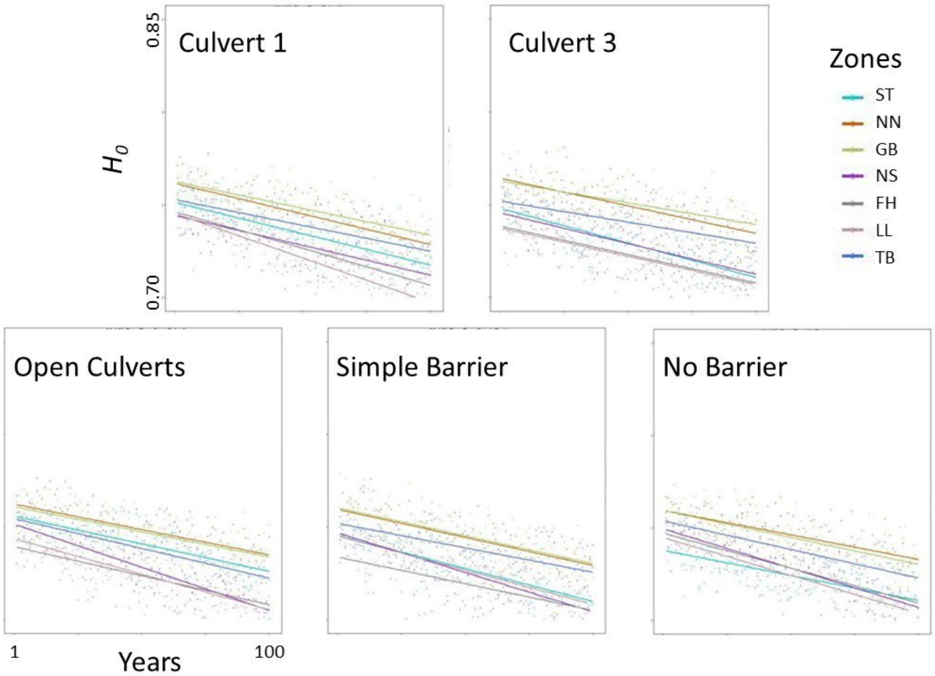


Figure 32 - Lake Mead area heterozygosity (H_o) over time by zone for each scenario. Zones are St Tomas (ST), Northshore North (NN), Gold Butte (GB), Northshore South (NS), Fortification Hill (FH), Lake LV East (LL), and Temple Bar (TB).

Key takeaways from Lake Mead area simulations

Tortoises in all barrier simulations had similar demographics, and populations remained stable or were growing through the simulations. Urban growth was predicted to be minimal and largely only affected the North Zone. The roadway between Northshore North and South did appear to be a barrier to movement, and this is likely due to the large fencing effort to keep tortoises off roadways. There are, However, a large number of culverts between the zones that create effective exchange between those two zones. The rivers serve to effectively separate zones from one another, and are largely barriers to exchanges of individuals and therefore gene flow. Taken together, these results could indicate that urbanization and linear barrier effects are relatively small, but weakly apparent, in this area of Clark County, Nevada. Culvert scenario recommendations based on simulation results:

- Overall, the Open Culverts or Culvert 1 scenario is recommended for the Lake Mead study area, as Culvert 3 is predicted to result in lower overall tortoise population numbers.
- Either the Open Culvert or Culvert 1 scenario is predicted to best maintain connectivity along Northshore Road.

Landscape: North Area

The North study area extends from North Las Vegas following I-15 northeast toward Mesquite, Nevada. The area also includes U.S. Highway 93 (US-93) extending northward from Apex through Coyote Springs. The zones closer to Las Vegas are the most heavily urbanized, and additional growth and solar development is expected to occur near Valley of Fire State Park (Figure 33).

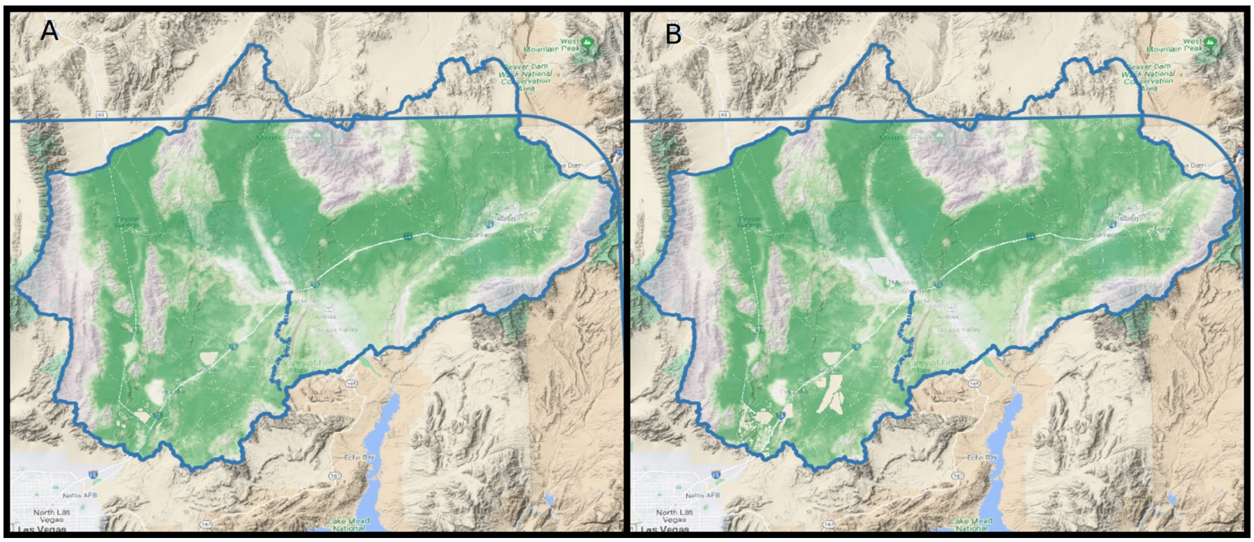


Figure 33 - The North study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020 and B) 2100.

There are six primary zones in the North area that were considered for analysis (Figure 34 and Table 5). These zones were separated by the larger roadways within the region (I-15, US-93, and State Route (SR) 168), as well as smaller routes (e.g. SR-169 and Carp-Elgin Road). Zonal areas had limited habitat reduction due to urbanization, with Moapa having the highest loss at 4% (31km) of the area. Habitat costs increased only slightly in the Moapa zone, and remained the same in the other five zones (Table 6).

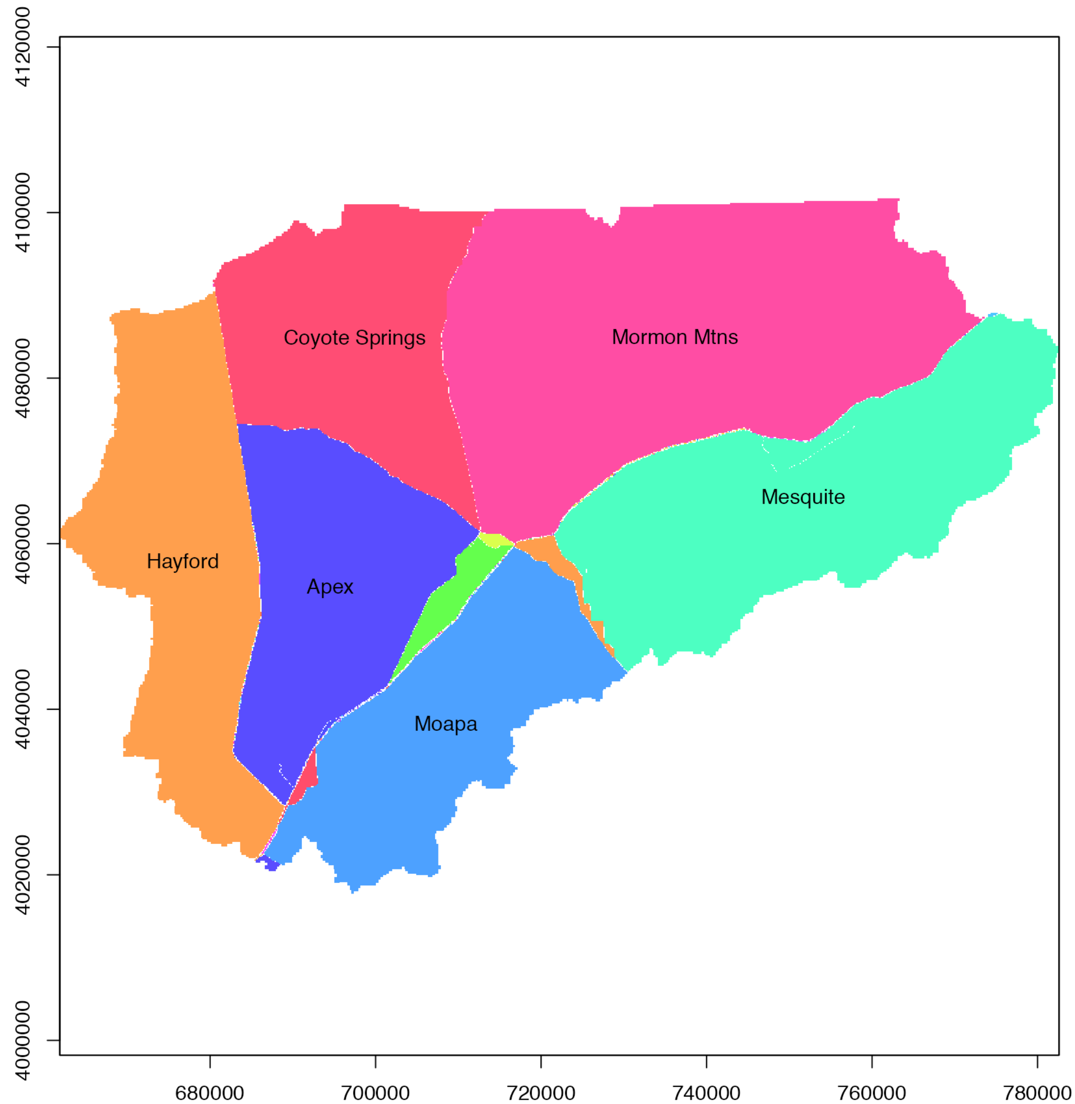


Figure 34 - Zones in the North study area that were used for analyses.

Table 5 - North area zones. Larger areas separated by prominent boundaries within the North area.

Zone	Zone Name	Description
11	Hayford	West of US-93, Hidden Valley
56	Apex	Apex triangle
2	Coyote Springs	North of SR-168 and I-15
1	Mormon Mtns	Mormon Mountains area
365	Moapa	South of I-15 to Moapa
13	Mesquite	South of I-15 to Mesquite

Table 6 - North area zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	Area (km^2)				Mean Cost			
	2020	2050	2100	Loss	2020	2050	2100	Change
Hayford	955.49	955.49	939.39	16.10	0.35	0.35	0.36	0.00
Apex	739.84	739.84	739.71	0.13	0.21	0.21	0.21	0.00
Coyote Springs	784.71	770.63	770.63	14.09	0.16	0.16	0.16	0.00
Mormon Mtns	1749.57	1740.64	1740.33	9.24	0.21	0.21	0.21	0.00
Moapa	749.26	719.53	717.56	31.70	0.22	0.22	0.22	0.01
Mesquite	1178.53	1178.44	1177.71	0.82	0.28	0.28	0.28	0.00

Demographics

Neither mortality rates (Figure 35) nor population numbers (Figure 36) appeared impacted by urban growth, or among the different scenarios within zones. The Hayford zone did appear to have declining numbers of individuals over time for culvert scenarios. In contrast, the Moapa Zone had increasing numbers of individuals for all scenarios (Figure 36).

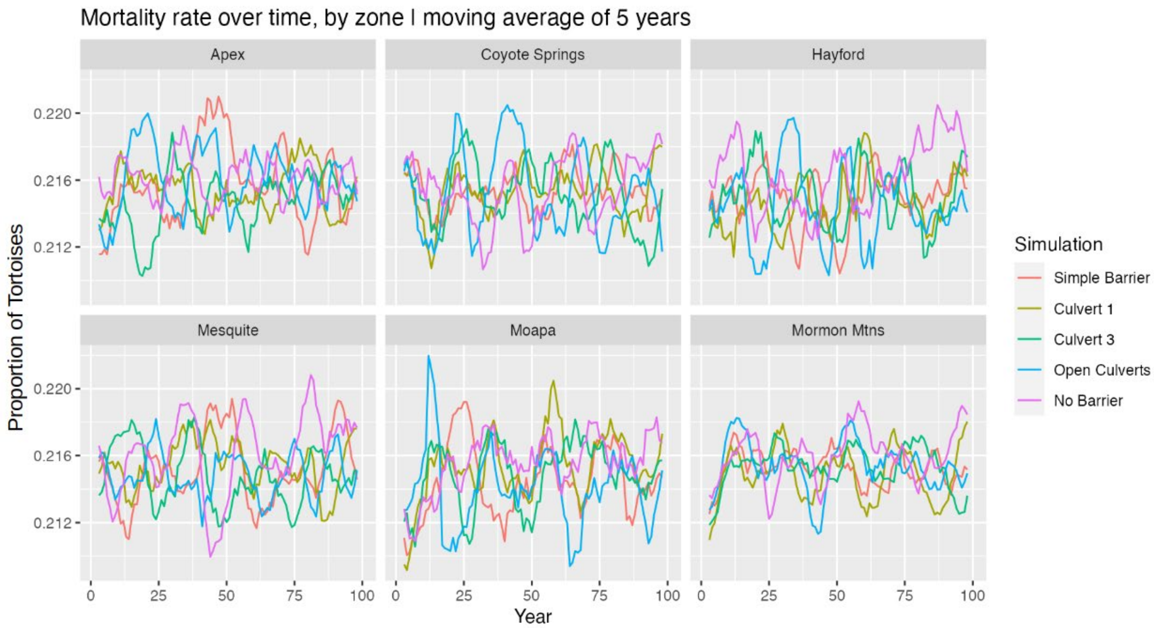


Figure 35 - Adult mortality rates in the North study area. Mortality proportions for adult tortoises are shown over time in each zone.

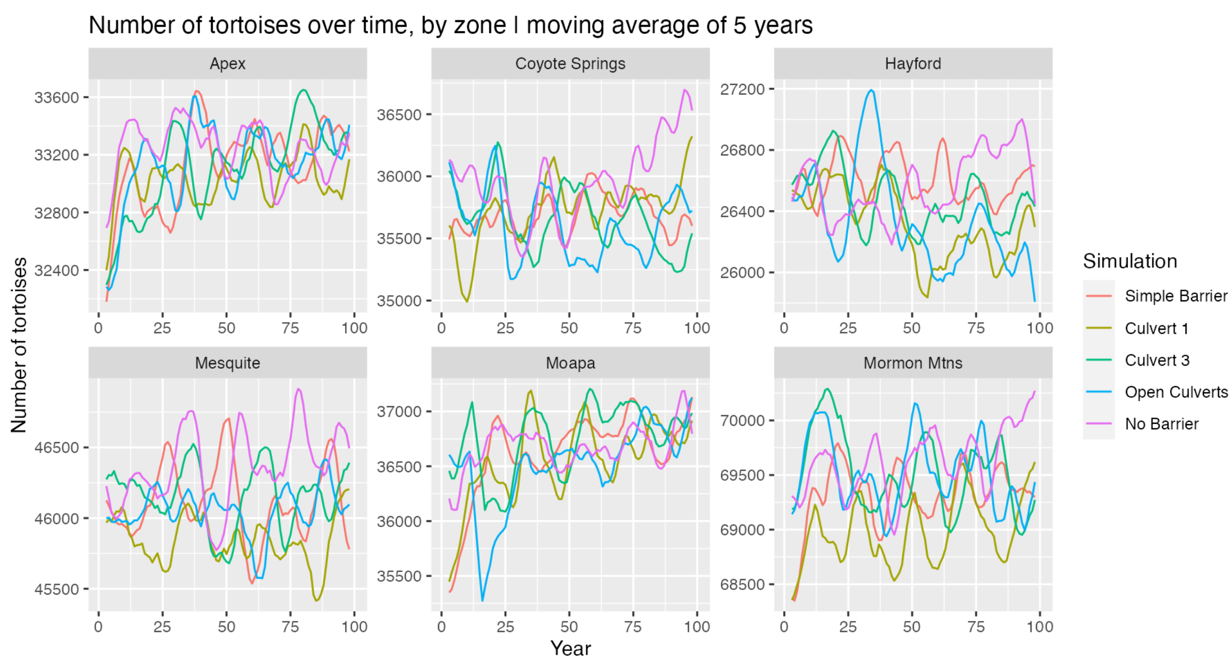


Figure 36 - Number of live tortoises in the North study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes.

The Coyote Springs and Apex zones exchanged the highest numbers of individuals in the culvert scenarios, while exchanges between Coyote Springs and Mormon Mtns, and Apex and Hayford were the next most connected (Figure 37). Crossings among zones near the town of Moapa were limited due to the interstate, with few culverts for crossing available. Crossings between Moapa and Mesquite were also very low, likely due the influence of the Muddy River (Figure 37). The culvert scenarios were predicted to have improved outcomes between several zones, similar to Open Culverts, when compared with the Simple Barrier (Figure 37). The barrier runs showed substantial reduction among zones, with the exception of Coyote Springs, as there are no culverts to differentiate crossings from that zone, and neither US-93 nor SR-168 qualified as a barrier to movement. Additional fencing could influence connectivity if not tied in with functional culverts. Immigration and emigration from zones indicated increasing levels (post burn-in) for culvert scenarios in the Apex and Coyote Springs zones over time (Figure 38). Levels among other zones remained largely unchanged.

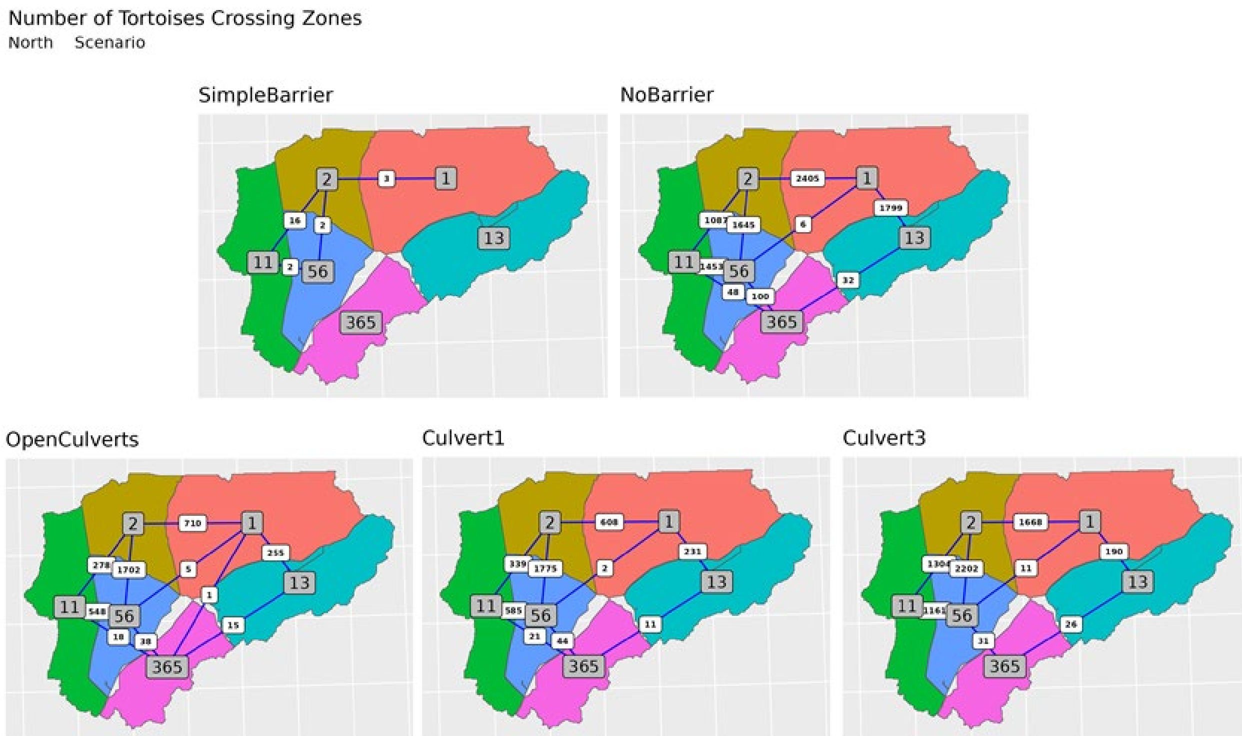


Figure 37 - The number of tortoises in the North study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels, and zone names are given in Table 5.

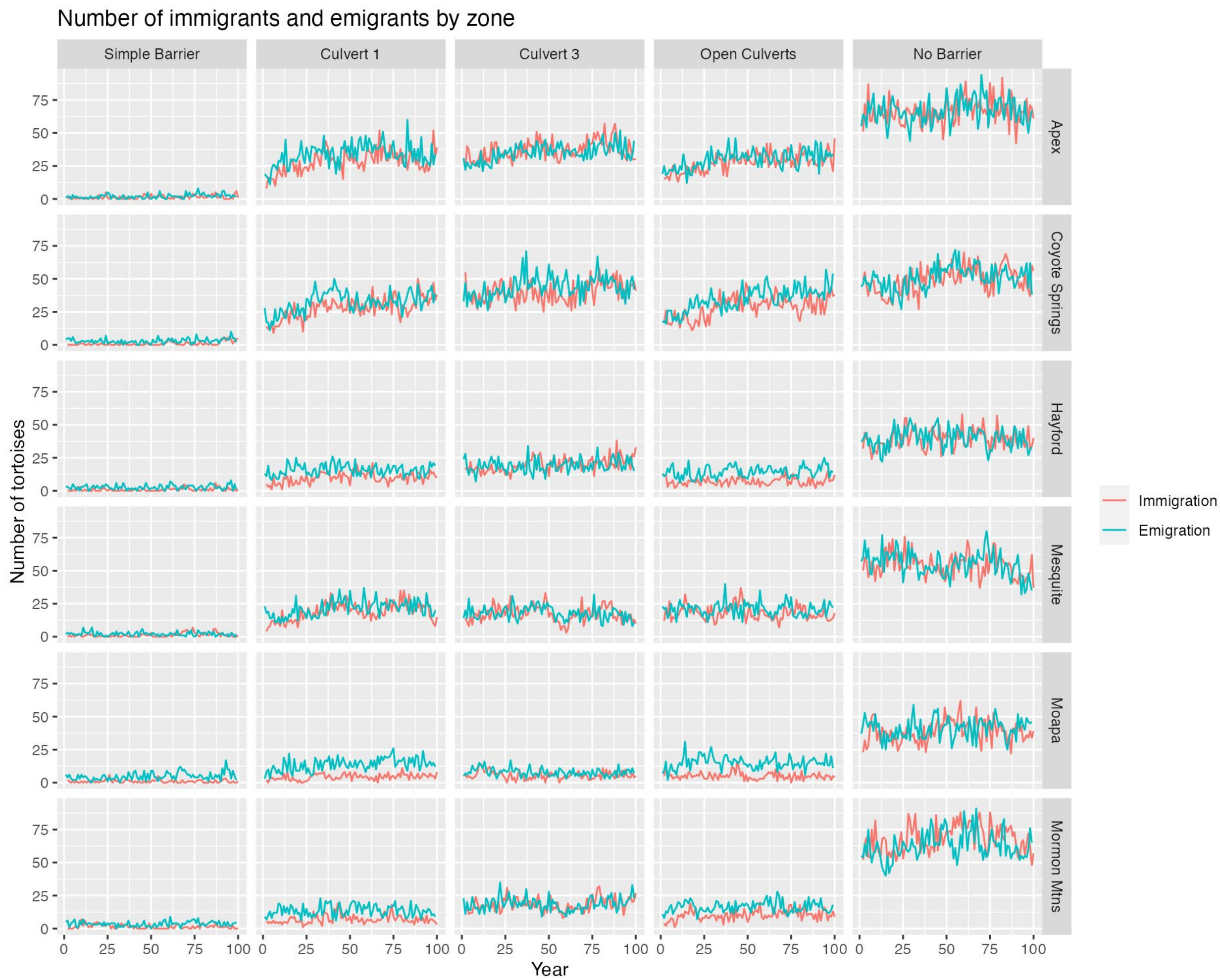


Figure 38 - Desert tortoise immigration and emigration in the North study area. Immigration and emigration are shown over time by zone for each scenario.

The number of tortoises mating between zones showed a reduction from a No Barrier scenario in culvert scenarios; however, Culvert 1 was predicted to maintain connectivity more similarly to the Open Culverts scenario, and better than Culvert 3 (Figure 39). The zones had differing levels of genetic exchange (via mating) between them, with comparable connectivity for culvert scenarios between Mormon Mtns and Coyote Springs, Coyote Springs and Hayford, and Coyote Springs and Apex (Figure 40). Connectivity was predicted to improve with the Culvert 1 scenario between Mormon Mtns and Mesquite, and Hayford and Apex (Figure 40). There were low levels of connectivity between Mesquite and Moapa, Apex and Moapa, Hayford and Moapa, and Mormon Mtns and Apex (Figure 40). The overall summary plot showed an initial increase in mortality rates that stabilized after 25 years, but all other metrics appeared similar among scenarios with the exception of mortality by density in the early years for the Open Culverts scenario, which was likely due to stochastic variation (Figure 41).

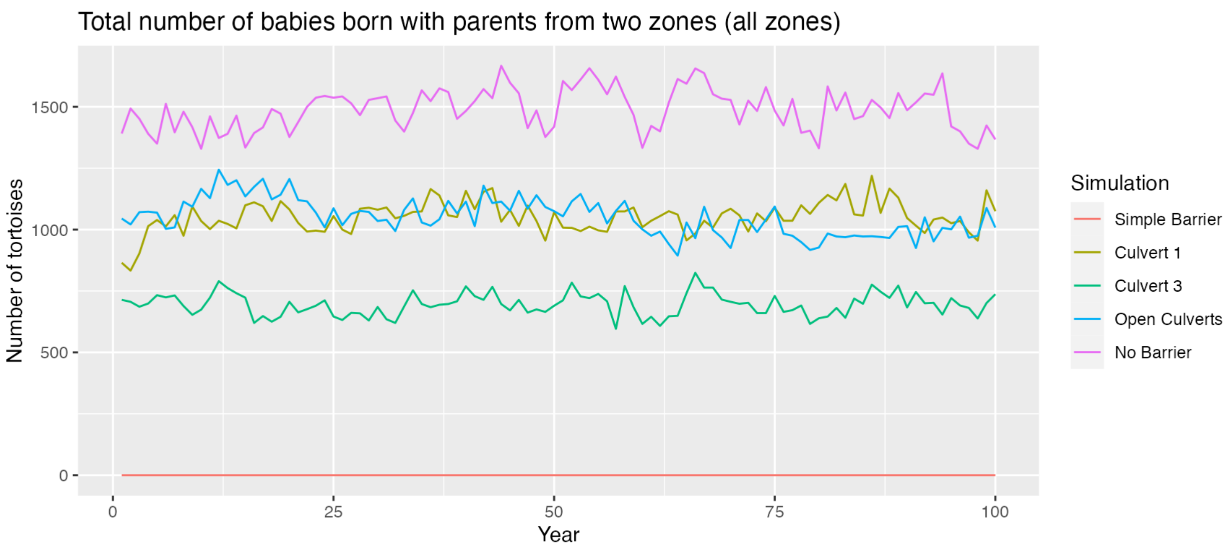


Figure 39 - Number of desert tortoises mating in the North study area. Mating is averaged across zones over time for each scenario.

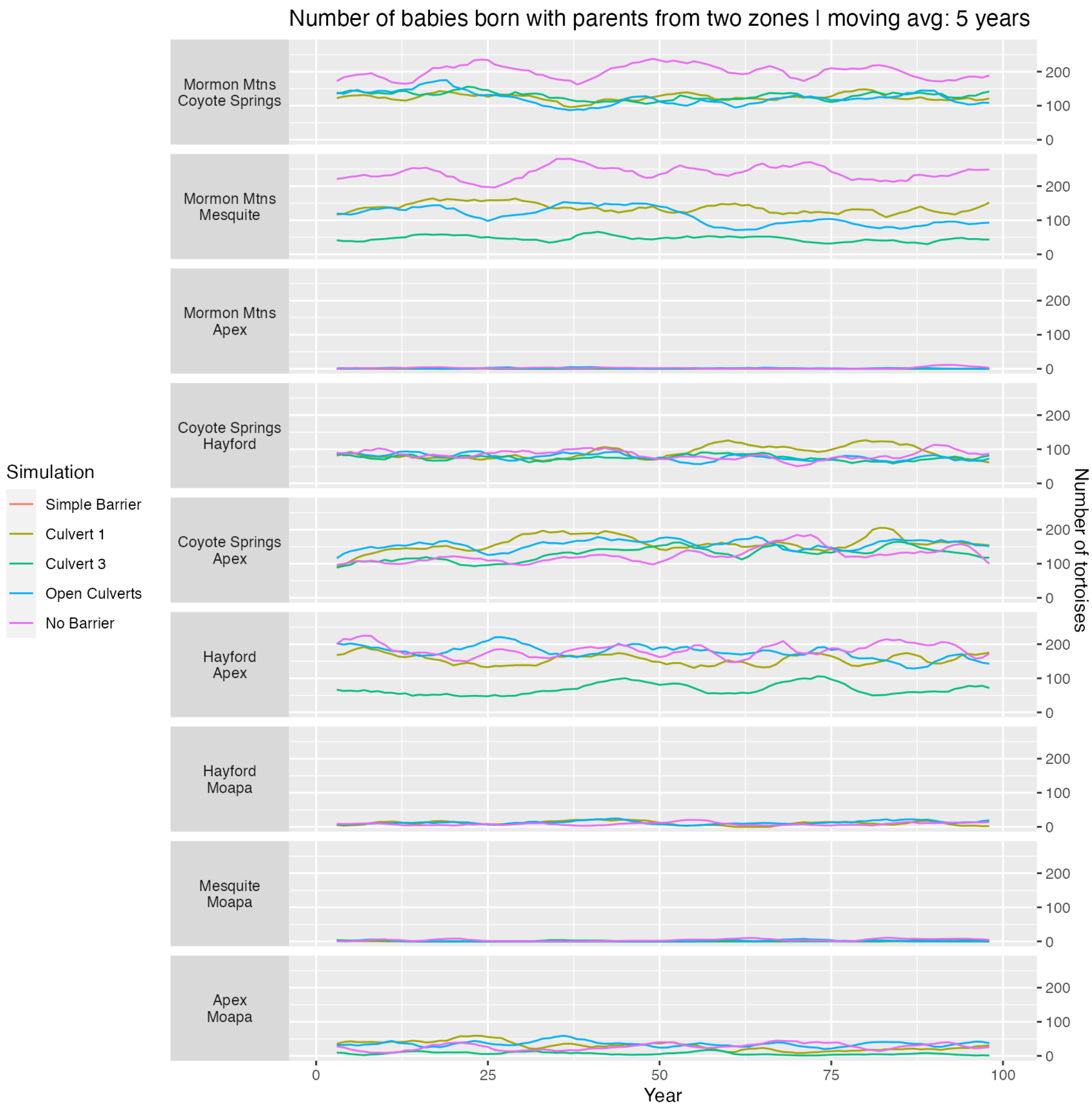


Figure 40 - North study area moving average of the number of offspring with parents originating in adjacent zones. Average values are displayed over time by zone for each scenario.

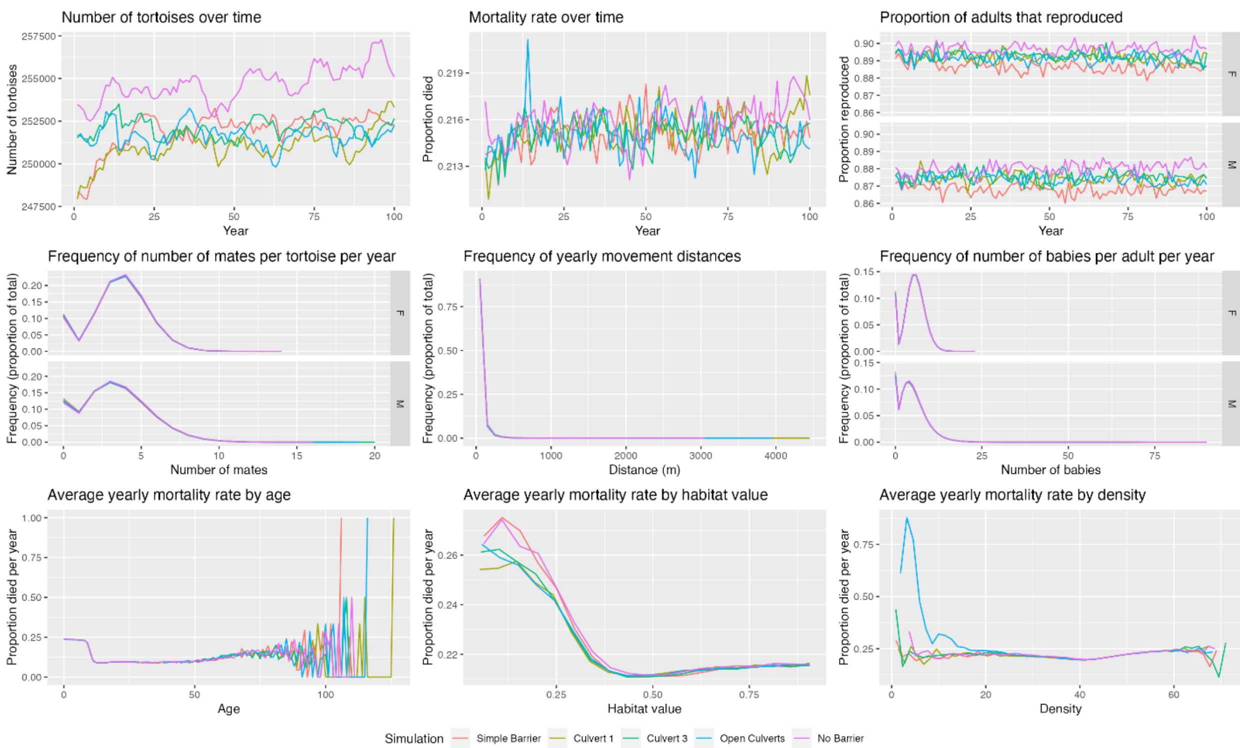


Figure 41 - North study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Of 20 microsatellite loci 12 to 20 were out of HWE by year 100; therefore, caution should be exercised when interpreting results where HWE is assumed. There was no evidence of IBD in any scenario (p-value > 0.05), indicating panmixia (Figure 42). Additionally, spatial analysis (sPCA) supported a genetic cline for all scenarios (p-value > 0.05). Genetic diversity did not reveal any additional information by scenario or zone; however, values were generally high, stable, and similar (Figures 43 and 44).

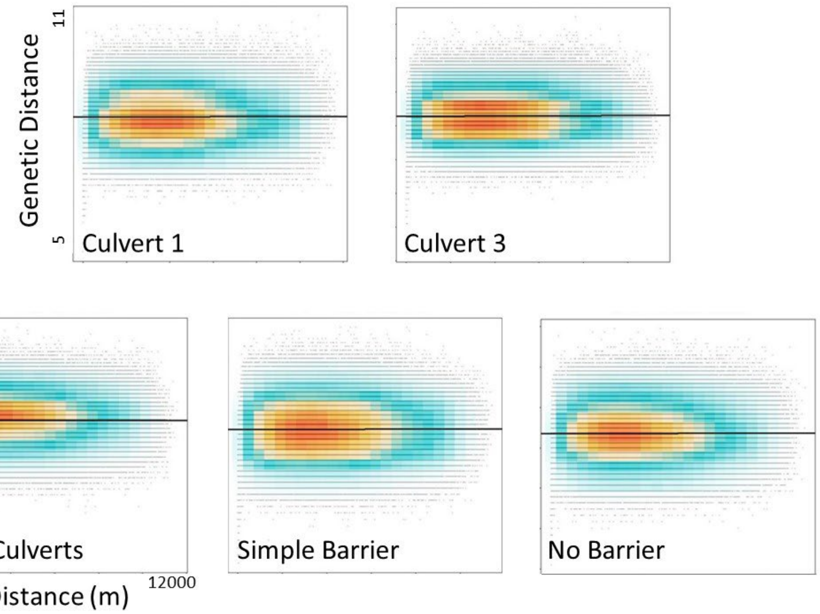


Figure 42 - North study area two-dimensional kernel density estimation for each scenario. The kernel density estimation colors represent the relative density of points (warmer colors signify higher densities) and the line shows the correlation between genetic and geographic distances.

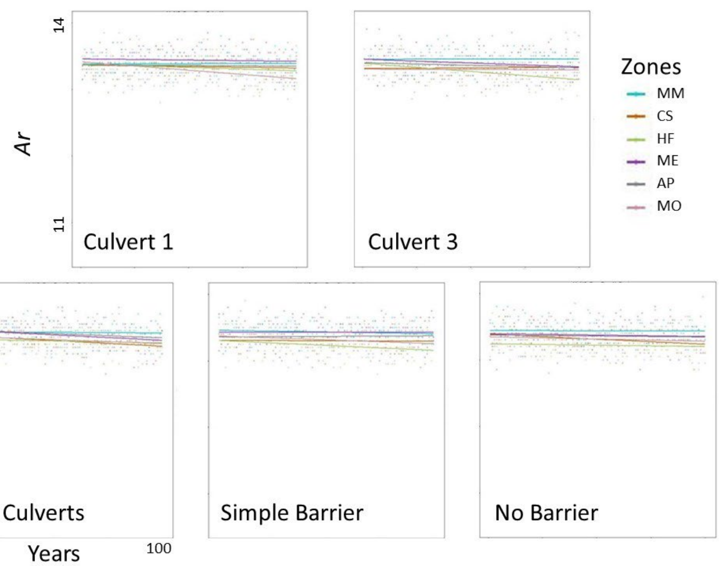


Figure 43 - North study area allelic richness (*Ar*) over time by zone for each scenario. Zones are Mormon Mountains (MM), Coyote Springs (CS), Hayford (HF), Mesquite (ME), Apex (AP), and Moapa (MO).

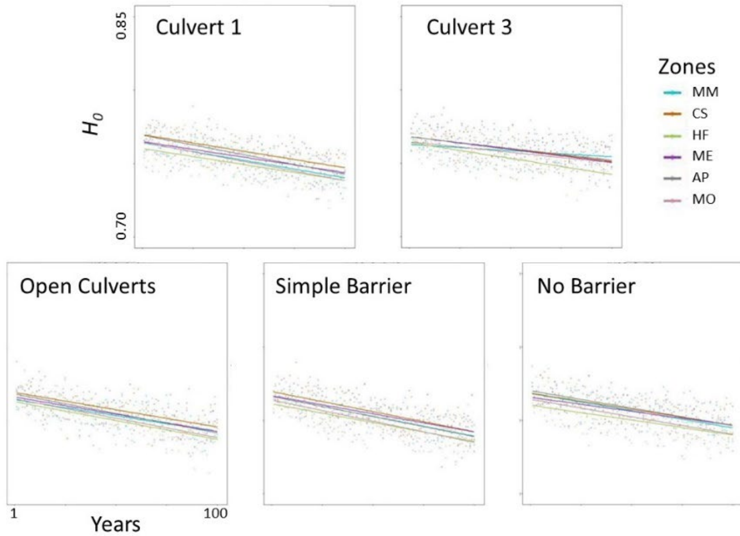


Figure 44 - North study area heterozygosity (H_o) over time by zone for each scenario. Zones are Mormon Mountains (MM), Coyote Springs (CS), Hayford (HF), Mesquite (ME), Apex (AP), and Moapa (MO).

Key takeaways from North area simulations

While there was measurable development in the North area, there were few changes detected in the mortality rates, or reductions in connectivity beyond those imparted by the corridors outright. I-15 remains a substantial barrier and there is little exchange between the zones on the west side of the interstate with those in the east. Further there is little exchange among the zones that are to the east of the interstate due to the effects of the Muddy River. The barrier scenarios showed significant reductions in connectivity, and this was most evident between the Mormon Mtns and Mesquite zones. Culvert scenario recommendations based on simulation results:

- The Open Culverts, or Culvert 1 scenario is recommended for maintaining connectivity overall across the study area.
- Specifically, the Open Culverts or Culvert 1 scenario is predicted to best improve connectivity outcomes along US-93 and the I-15 north of Las Vegas, Nevada. In contrast,

the current conditions scenario (Culvert 3) showed reduced connectivity between zones bisected by I-15 (Mormon Mtns and Mesquite; Hayford and Moapa; Apex and Moapa) relative to the other culvert scenarios.

Landscape: Northwest Corridor

The Northwest corridor extends from northwest Las Vegas following U.S. Highway 95 (US-95) north of the Spring Range passing through Indian Springs, and encompassing much of the Nevada Test Site, the Nellis Bombing range, and the U.S. Fish and Wildlife Service Desert Refuge within Clark County, Nevada. The zones closer to the city are subject to current and predicted future urbanization. Extending to the Northwest, tortoise habitat is divided by a major highway (US-95) and north of the highway are extensive military training areas. Urban growth is projected to continue in northern portion of the Las Vegas Valley (Figure 45).

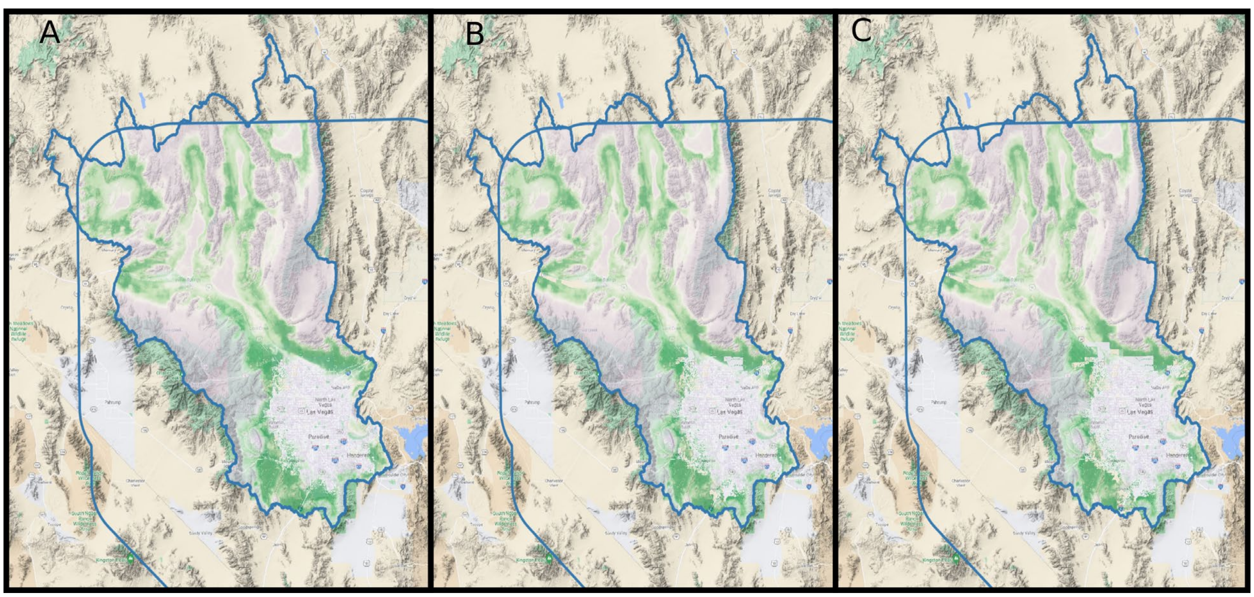


Figure 45 - The Northwest corridor study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020, B) 2050, and C) 2100.

The Northwest corridor was divided into eight primary zones for analysis (Figure 46 and Table 7). These areas are separated by key barriers in the region (e.g. US-95 and I-15) as well as smaller roadways (e.g. Kyle Canyon Road, Lee Canyon Road, and Lake Mead Boulevard) that bisect tortoise habitat. Zonal area was expected to decrease through time in zones that were predicted to have urban growth. Areas were reduced by 9 to 18% in the four zones most heavily impacted by urban growth across the simulation (Sloan, East LV, Spring Mtns, and Blue Diamond. Habitat costs increased in six of the eight zones, indicating that the habitat lost was on average lower in cost (i.e. had higher habitat value). This is due to the overlap of new development with tortoise habitat (Table 8).

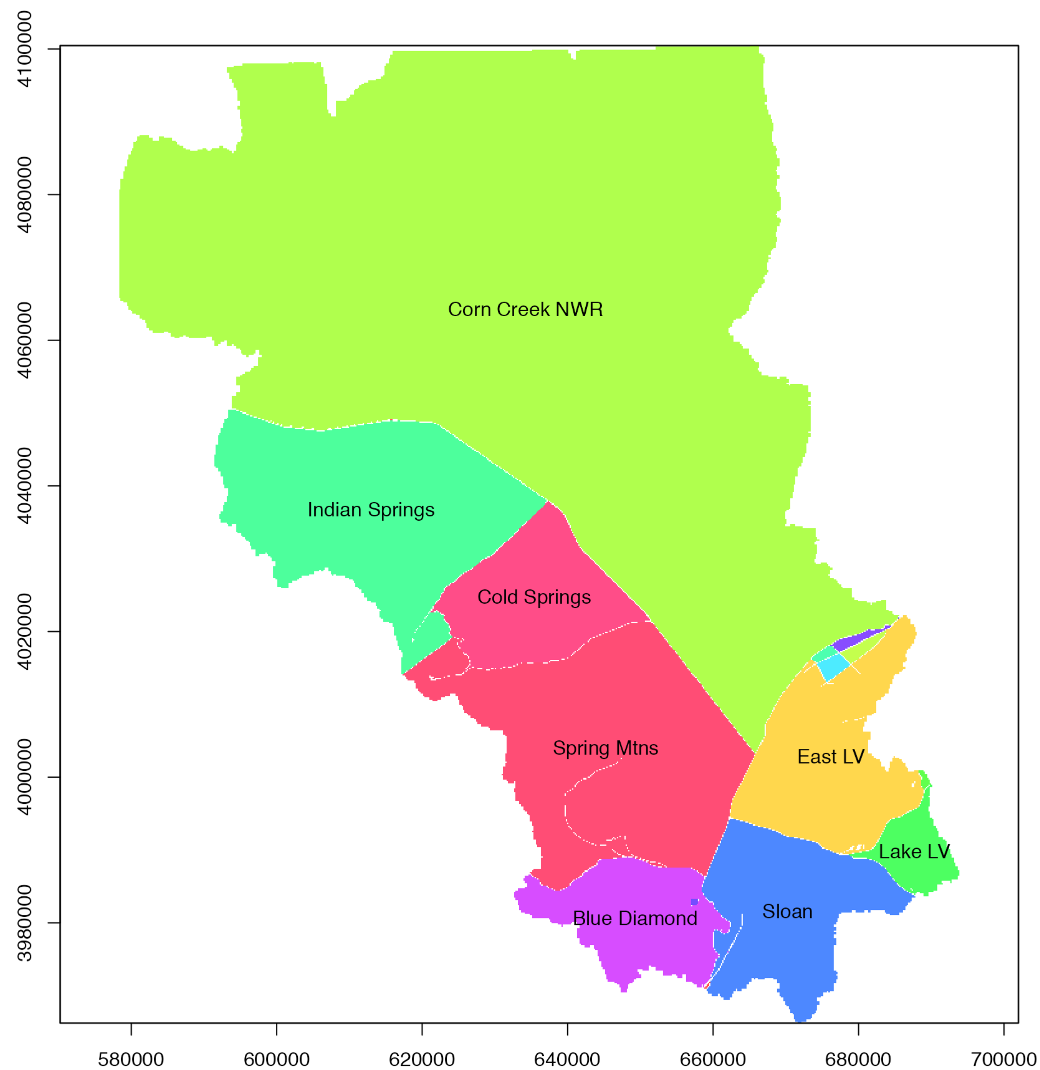


Figure 46 - Zones in the Northwest corridor study area that were used for analyses.

Table 7 - Northwest corridor zones. Larger areas separated by prominent boundaries within the Northwest corridor area. (Note that NWR is being used as an abbreviation for National Wildlife Area).

Zone	Zone Name	Description
54	Sloan	South Las Vegas, Sloan
53	Blue Diamond	Blue Diamond
40	Spring Mtns	Spring Mountains, west Las Vegas
29	Cold Springs	Prison area
30	Indian Springs	North Spring Mountains, bajada
33	Corn Creek NWR	Nevada National Test Site
41	Lake LV	Lake Las Vegas
38	East LV	East Las Vegas

Table 8 - Northwest corridor zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	Area (km^2)				Mean Cost			
	2020	**2050**	**2100**	**Loss**	**2020**	**2050**	**2100**	**Change**
Sloan	335.52	295.45	273.61	61.92	0.38	0.42	0.44	0.05
Blue Diamond	311.40	304.14	288.52	22.89	0.26	0.26	0.27	0.01
Spring Mtns	793.52	754.09	721.53	71.98	0.48	0.49	0.51	0.03
Cold Springs	364.28	361.06	351.19	13.09	0.47	0.47	0.48	0.01
Indian Springs	866.73	837.12	837.12	29.61	0.51	0.51	0.51	0.01
Corn Creek NWR	5225.31	5181.38	5125.75	99.56	0.45	0.45	0.45	0.00
Lake LV	83.09	82.03	80.60	2.49	0.32	0.32	0.32	0.00
East LV	258.81	252.34	229.05	29.76	0.49	0.49	0.51	0.02

Demographics

Changes in area and habitat resulted in changes in demographics over time. Adult death rates largely remained near 21 to 22% with underlying random fluctuations, except when development occurred within zones. For example, mortality rates increased noticeably in East LV because of urbanization, and in the culvert scenarios in the Spring Mtns at around year 60 because of urbanization and in Indian Springs at roughly year 25 because predicted utility-scale solar development (Figure 47). Over the same time frame the Culvert 1 and Open Culverts scenarios saw population drops at Indian Springs ca year 25, and East LV and Sloan showed a gradual reduction from year 60 onward, while Corn Creek showed marked declines at both time periods (Figure 48). Zones that retained stable population levels were the Spring Mtns, Lake LV, and Cold Springs.

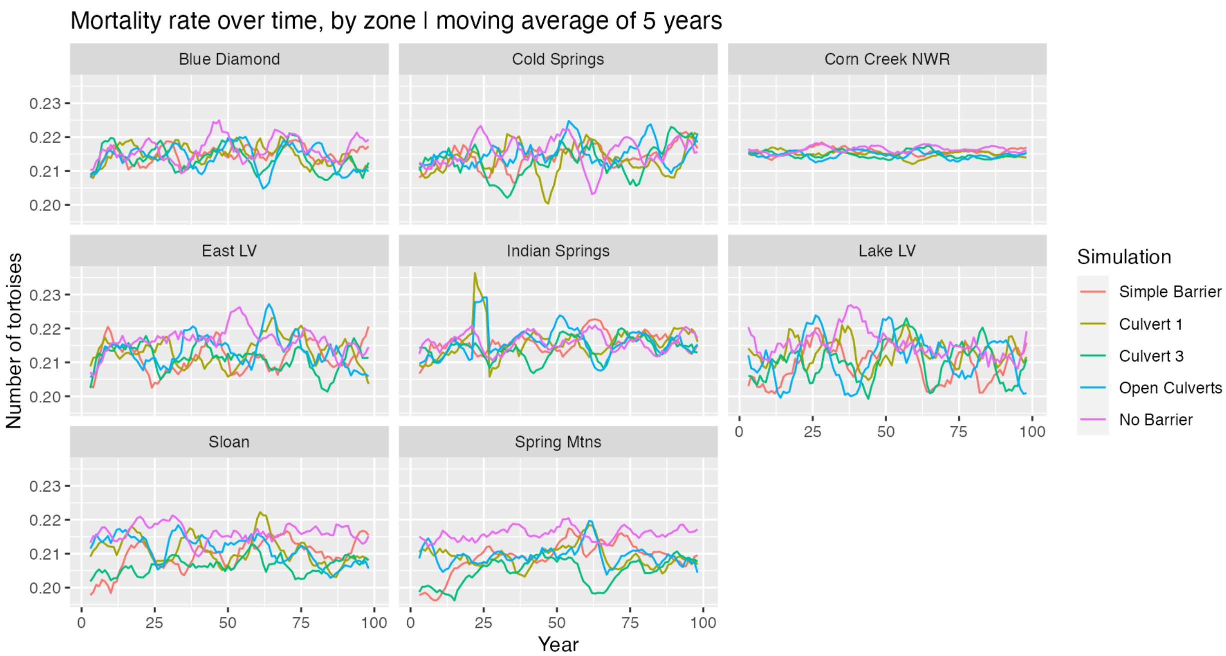


Figure 47 - Adult mortality rates in the Northwest corridor study area. Mortality proportions for adult tortoises are shown over time in each zone.

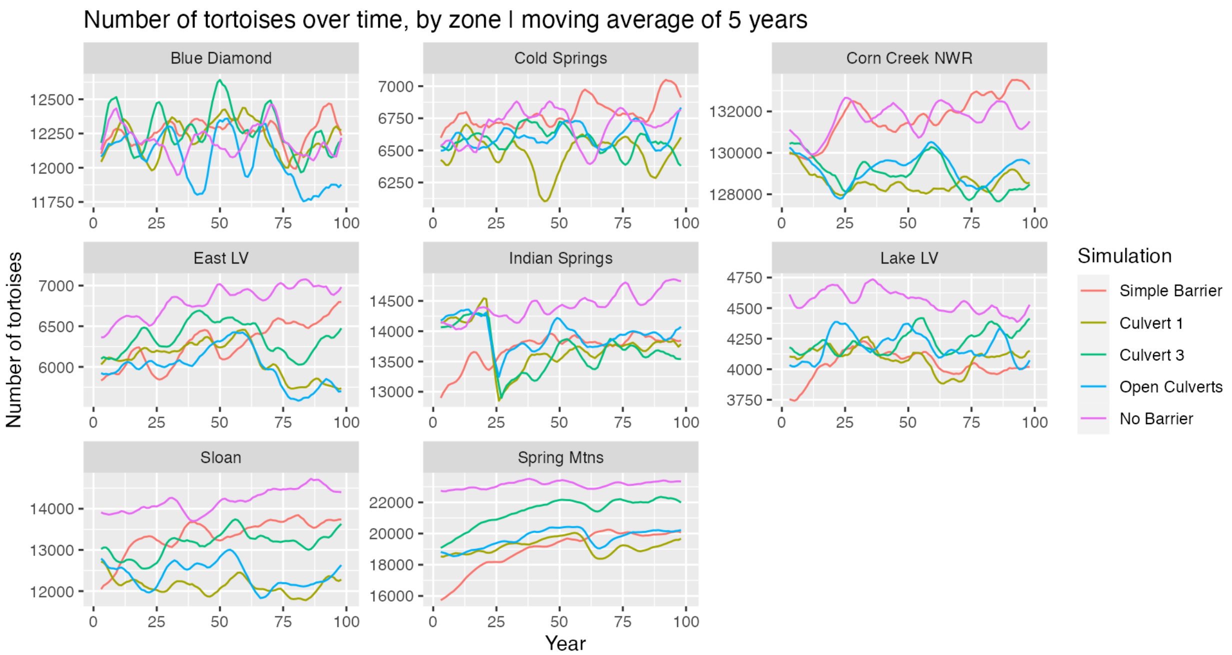


Figure 48 - Number of live tortoises in the Northwest corridor study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes.

Movement was spatially constrained by the locations of culverts in combination with the habitat areas within the region that influenced tortoise density. The footprint of the Las Vegas metropolitan area has resulted in a partial ring of habitat in the southeast and mountains in the have created a natural corridor in the central portion of the study area. Therefore, the configuration of available tortoise habitat differs from the configuration of the zone map (Figure 49). The map depicting movements among zones showed the most movement between Corn Creek, Indian Springs, and Cold Springs, and to a lesser extent Spring Mtns, Blue Diamond, and Sloan (Figure 50). There were few tortoises and culverts in the southern extent of the US-95 corridor, which was also an area of higher urbanization and growth over time (Figure 50). Thus, the expectation would be a reduced exchange of individuals between those zones. The barrier runs showed differential reduction among zones, where there was a 75% drop from the No Barrier configuration to the barrier runs between the Spring Mtns and Blue Diamond zones, while Cold Springs to Corn Creek showed a reduction of only 50%. Cold Springs to Indian Springs showed almost no reduction at all. Two sets of zones showed reductions in rates for the Open Culverts relative to the Culvert 1 scenario: Cold Springs to Indian springs and Indian Springs to Corn Creek. Zone specific Immigration/emigration showed a broad pattern of general reduction over time for the Indian Springs and Spring Mtns zones (Figure 51). Sloan showed almost no connectivity among the other zones and Lake LV had slightly higher but very limited immigration/emigration.

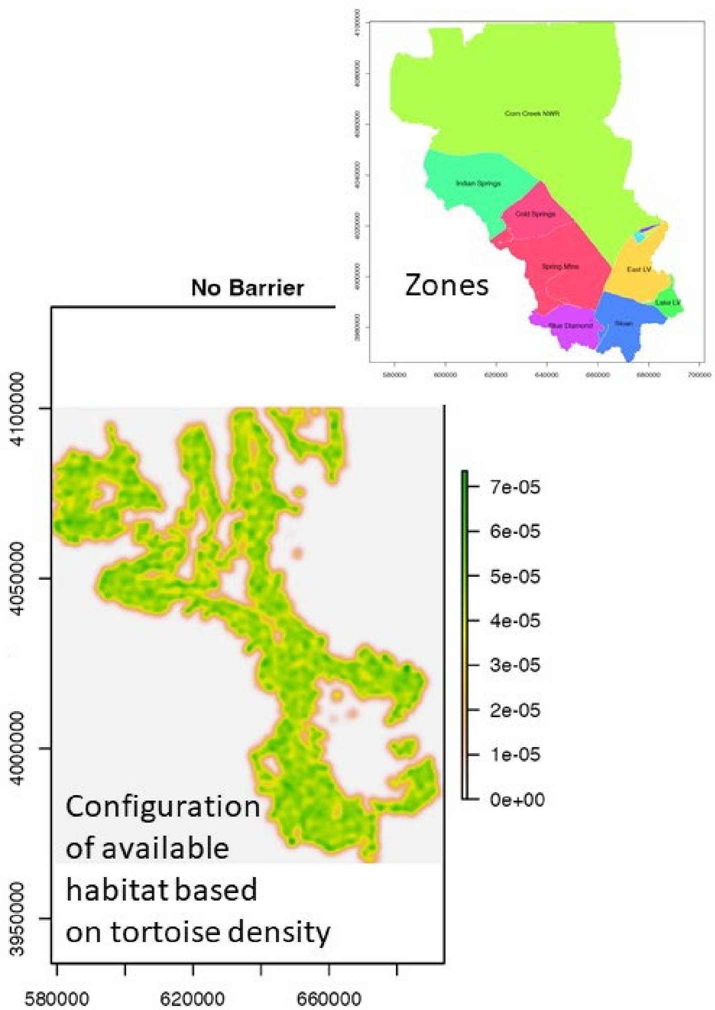


Figure 49 – Configuration of available habitat based on predicted tortoise density in the Northwest corridor study area, which differs from the zone map. Urbanization in the southeast has created a partial ring of habitat and mountains in the central portion have formed a natural corridor.

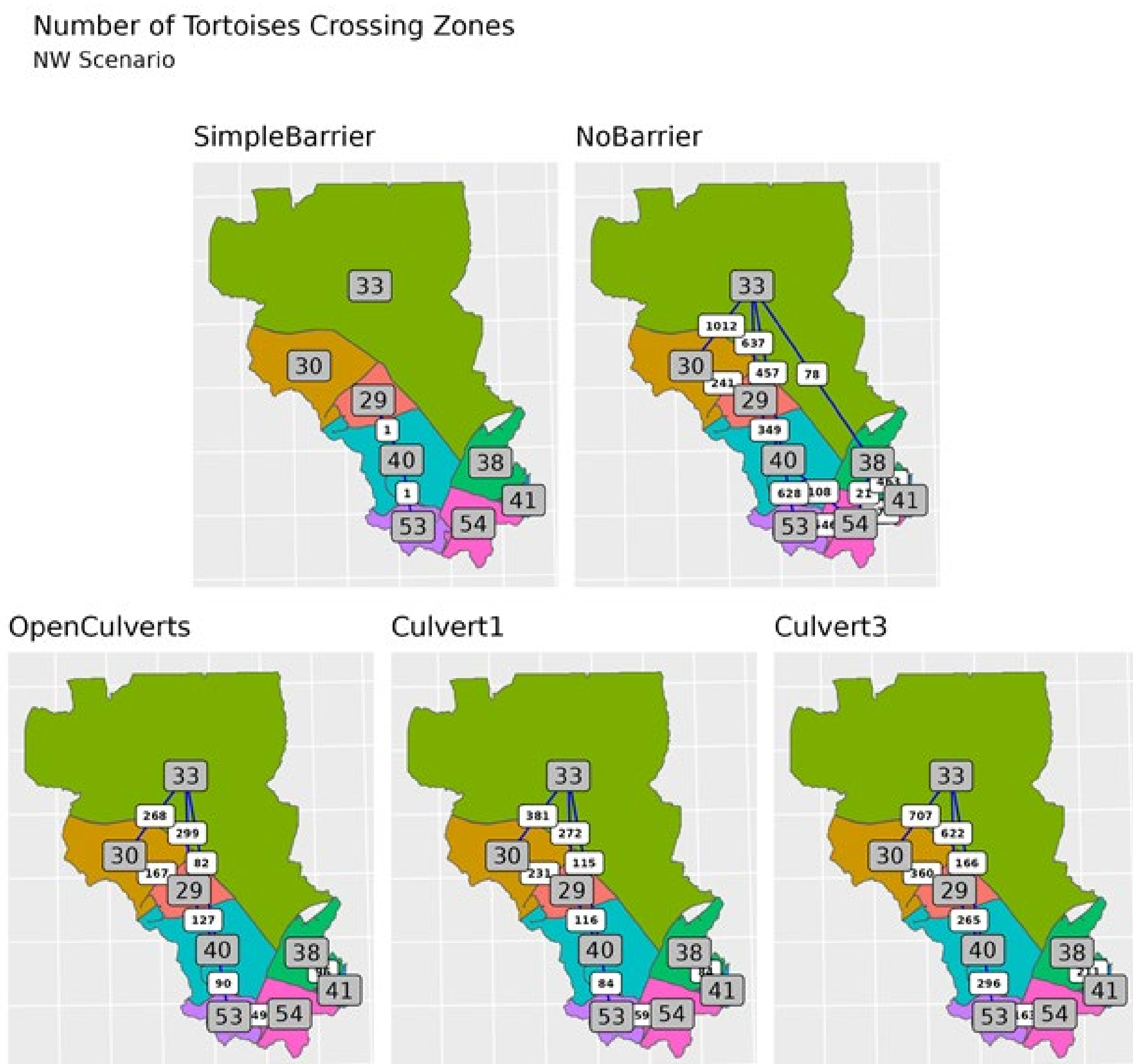


Figure 50 - The number of tortoises in the Northwest corridor study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels, and zone names are given in Table 7.

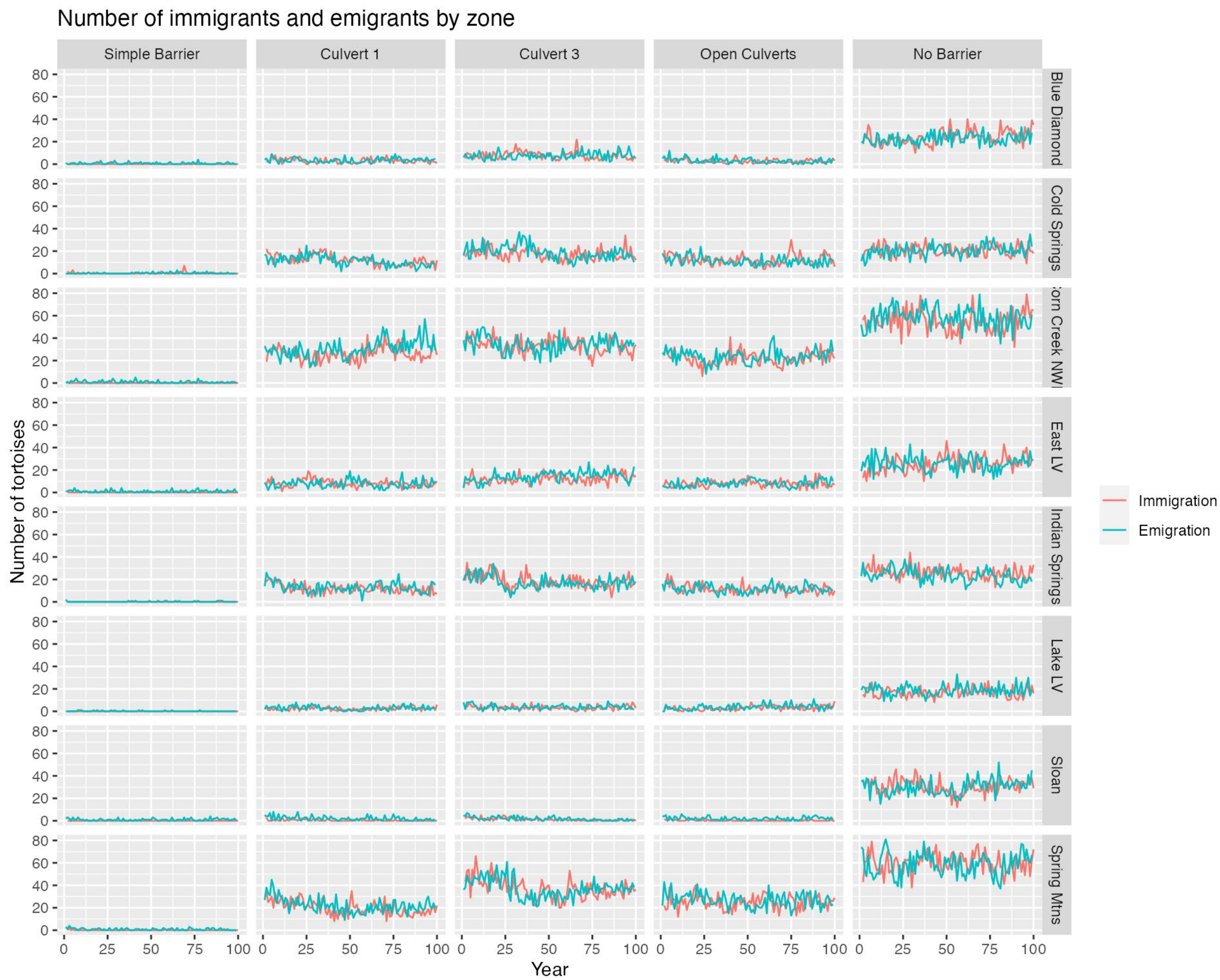


Figure 51 - Desert tortoise immigration and emigration in the Northwest corridor study area. Immigration and emigration are shown over time by zone for each scenario.

Culvert scenarios showed reduced cross-zonal reproduction relative to a No Barrier situation and overall there was a downward trend in reproduction across zones in culvert scenarios beginning after roughly 20 years (Figure 52). Culvert 3 may maintain connectivity better than Culvert 1; however, there is considerable overlap between the two and any culvert scenario (Open Culverts, Culvert 1, Culvert 3) was preferable to a Simple Barrier scenario, where connectivity is predicted to be lost entirely (Figure 52). The number of tortoises mating between zones reflected a similar pattern with culvert scenarios performing comparably (Figure

53). The summary plot for this region showed similar performance among zones for most parameters (Figure 54).

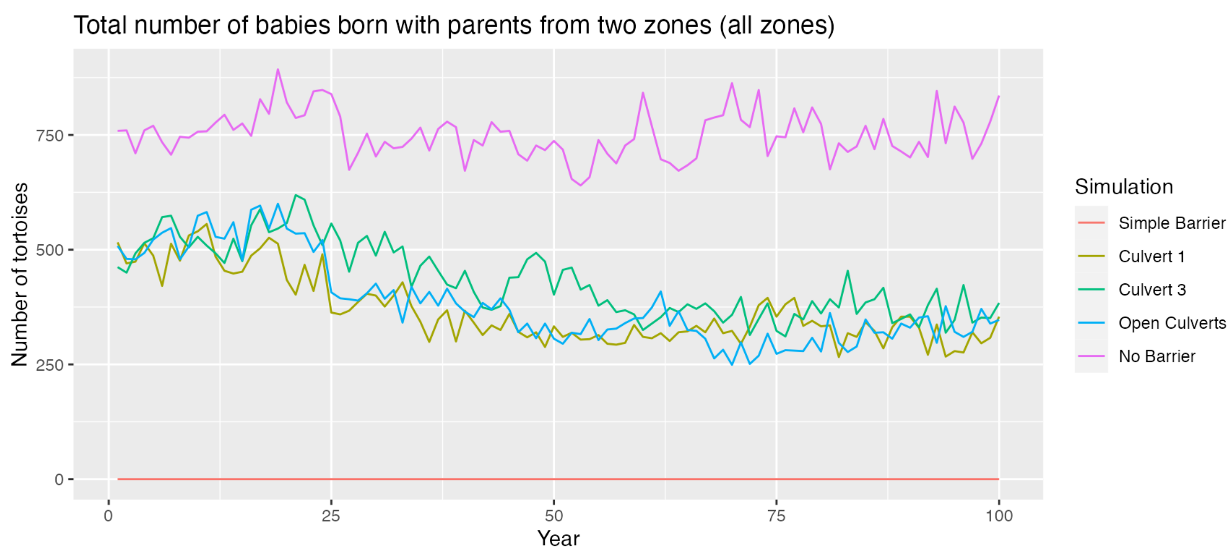
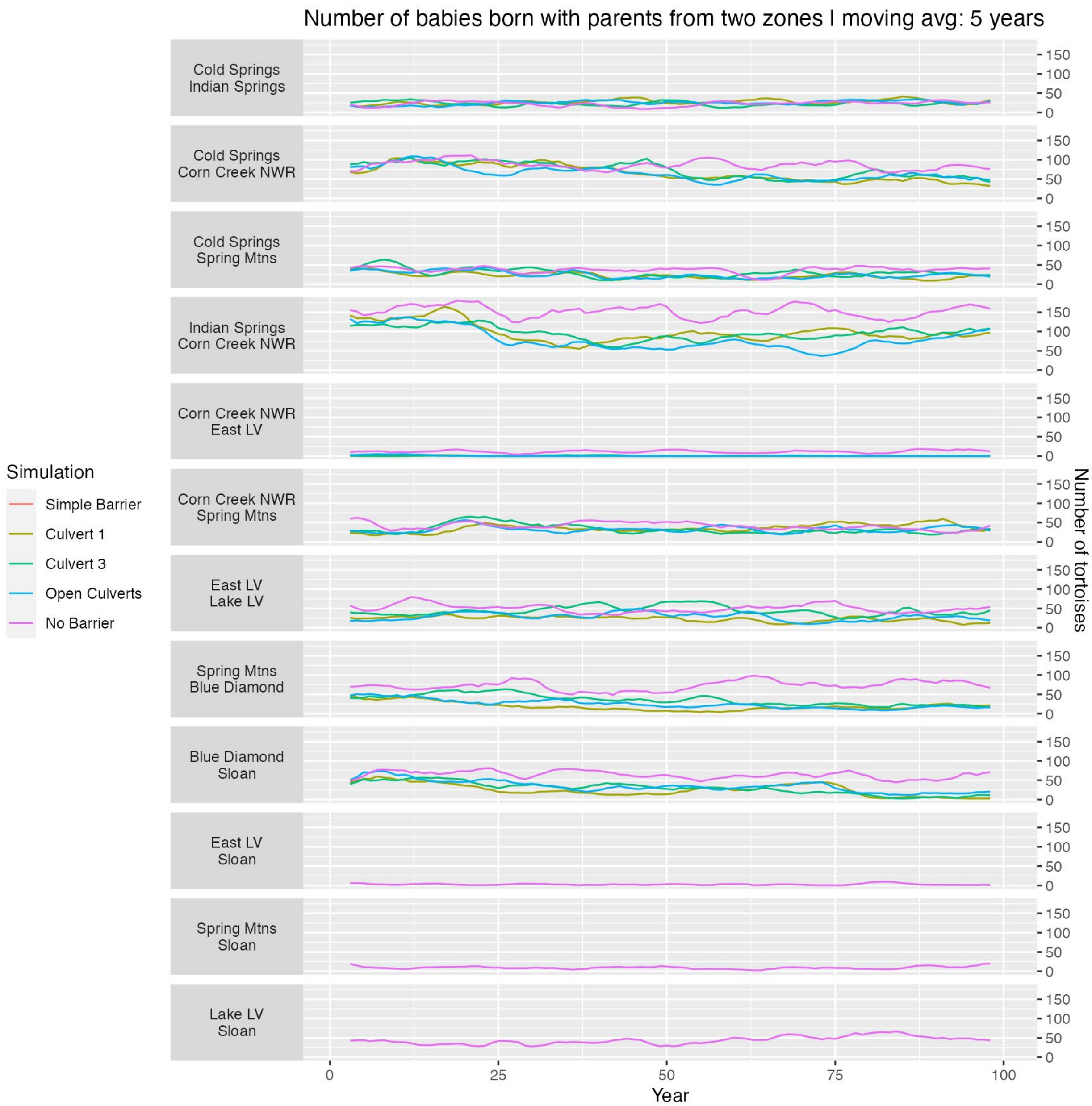


Figure 52 - Number of desert tortoises mating in the Northwest corridor study area. Mating is averaged across zones over time for each scenario.



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1085 Figure 53 - Northwest corridor study area moving average of the number of offspring with
1086 parents originating in adjacent zones. Average values are displayed over time by zone for each
1087 scenario.

1088

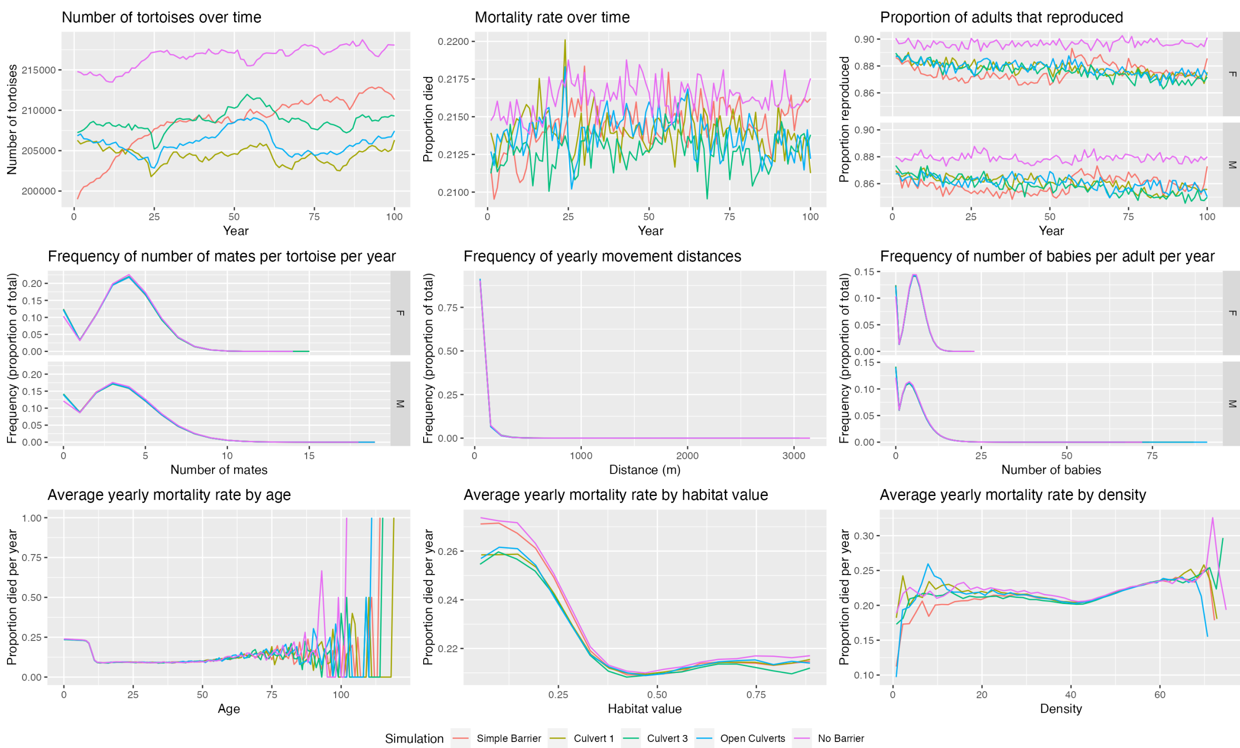

Figure 54 - Northwest corridor study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Although F_{ST} appeared to increase through time in all scenarios, it was only found to increase significantly in the disturbance scenarios (Open Culverts, Culvert 1, Culvert 3, Simple Barrier; *p*-value < 0.05). Zones in these scenarios appeared to be impacted, including: Indian Springs and Lake LV in all disturbance scenarios and Sloan in the Open Culverts and Culvert 1 scenarios (Figure 55).

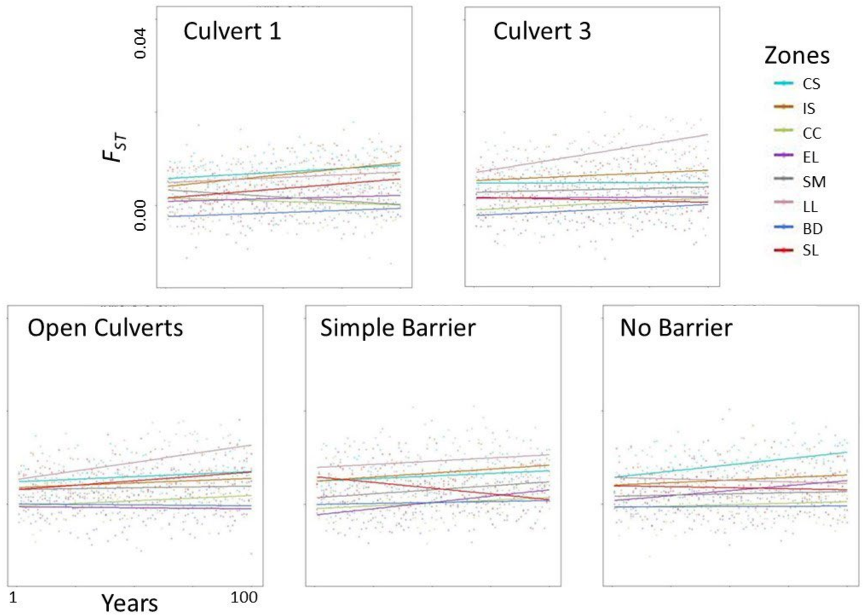

Figure 55 - Northwest corridor study area genetic differentiation (F_{ST}) over time by zone. Zones are Cold Springs (CS), Indian Springs (IS), Corn Creek NWR (CC), East LV (EL), Spring Mtns (SM), Lake LV (LL), Blue Diamond (BD), and Sloan (SL).

Key takeaways from Northwest corridor simulations

The Northwest corridor has a substantial increase in development predicted within the 100-year modeling scenario. The zones closest to the boundaries of the current Las Vegas metropolitan area show the largest impacts to desert tortoises, including reductions in population sizes predicted in the Sloan, East LV and Corn Creek zones. Connectivity is significantly reduced in areas nearest to urban areas, isolating some zones from one another completely. Over time zones associated with urban areas are predicted to become increasingly fragmented from adjacent zones. It is likely that habitat loss coupled with linear barriers will have a greater impact on connectivity than barriers alone in the Northwest corridor. Because habitat loss has a large impact on this area and is predicted to increase, habitat amount and configuration have been impacted. Any efforts to offset or reduce planned or future development in this area, or restrict it to already disturbed areas, are highly recommended

Culvert scenario recommendations based on simulation results:

- The Culvert scenarios are predicted to perform comparably in this study area; however, Culvert 1 or Open Culverts may provide the greatest connectivity benefits outside the city of Las Vegas.

Landscape: Pahrump Area

The Pahrump area encompasses Mesquite Valley, contains portions of Clark and Nye Counties, and borders Nevada and California on the western side of Clark County, Nevada. The study area includes the city of Pahrump in the north, as well as the smaller town of Sandy Valley, and dispersed urban/residential areas around the Ash Meadows and Amargosa areas. The study area is impacted by mining, roads (including a SR-160), OHV use, transmission line rights of way, etc. Solar and urban development are expected to occur in the area, and these appear on the simulated landscape around the year 2040 (Figure 56).

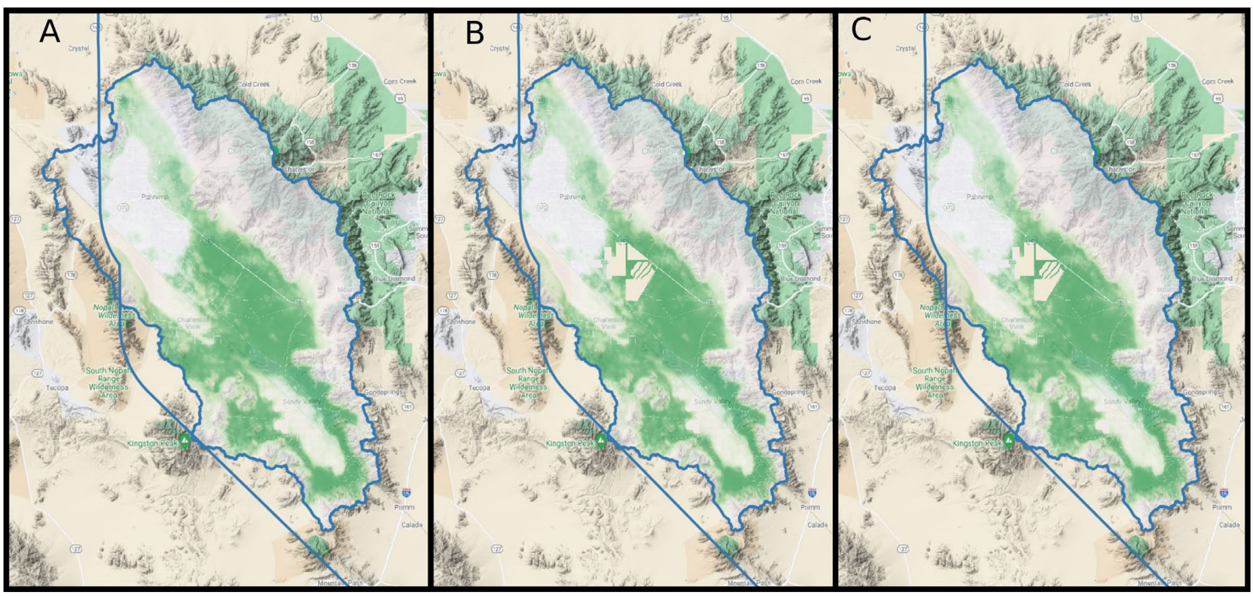

Figure 56 - The Pahrump study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020, B) 2050, and C) 2100.

The region was divided into three primary zones for analysis (Figure 57 and Table 9). These depict areas that are separated by SR-160 and SR-372, which connects Las Vegas and Death Valley National Park, respectively. The zone most impacted by future development was Pahrump West but Pahrump Pocket is currently the most developed. Pahrump West is projected to experience development that noticeably reduced habitat, resulting in the loss of 74 km^2 of habitat near SR-160. The other zones are less affected by urban growth, but are also composed of lower quality desert tortoise habitat (e.g. higher cost) than Pahrump West (Table 10). Overall, changes to average habitat quality in the Pahrump area were minimal, as the habitat areas were rather large.

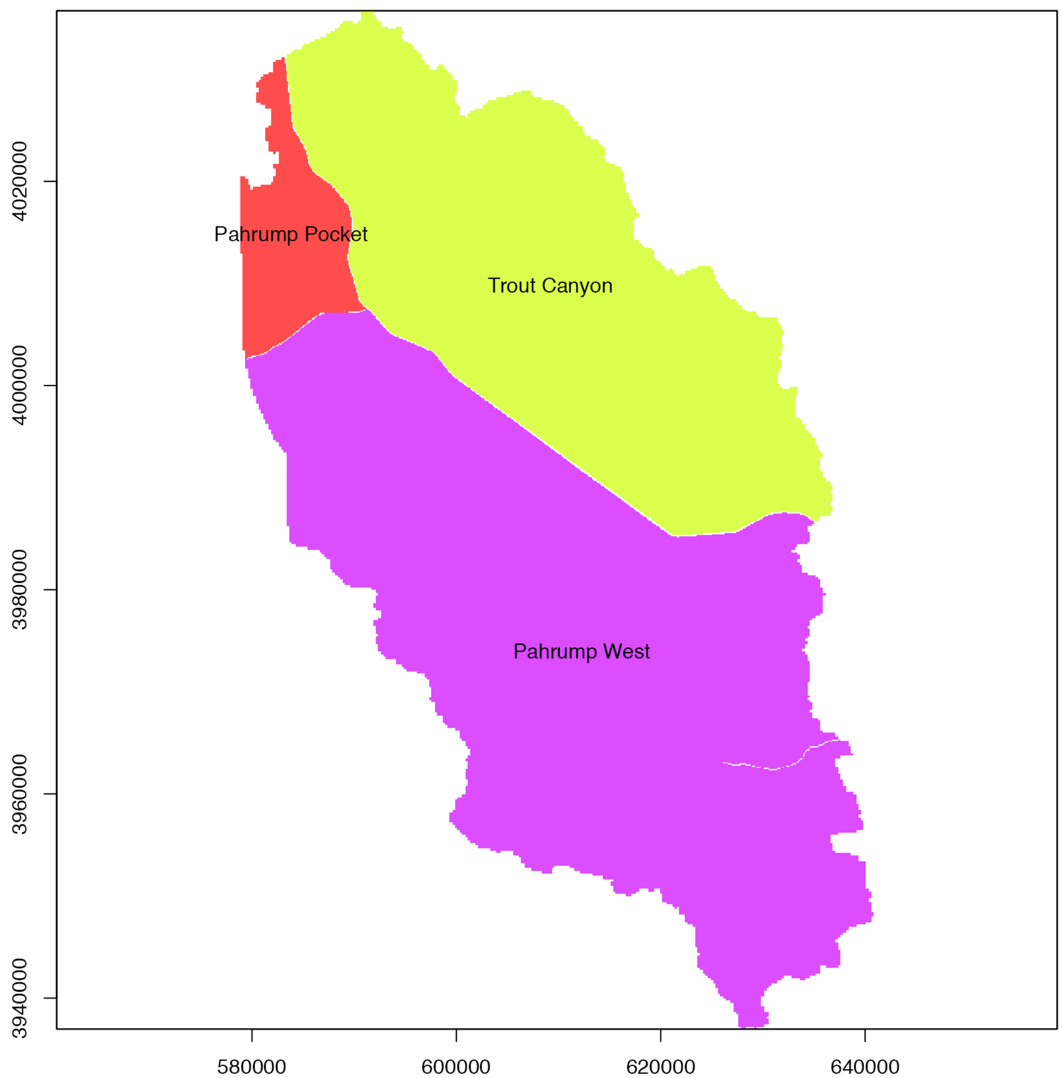

Figure 57 - Zones in the Pahrump study area that were used for analyses.

Table 9 - Pahrump zones. Larger areas separated by prominent boundaries within the Pahrump area.

Zone	Zone Name	Description
3	Trout Canyon	East of Pahrump and SR-160
4	Pahrump Pocket	Pahrump area and north
5	Pahrump West	West of SR-160

Table 10 - Pahrump zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	Area (km^2)				Mean Cost			
	2020	2050	2100	Loss	2020	2050	2100	Change
Trout Canyon	1304.66	1304.63	1304.52	0.14	0.46	0.46	0.46	0.00
Pahrump Pocket	165.23	165.04	164.63	0.60	0.47	0.47	0.47	0.00
Pahrump West	2013.77	1939.76	1939.51	74.26	0.28	0.29	0.29	0.01

Demographics

Changes in demographics over time were associated with projected growth following an increase in tortoise death rates after urban development (Figure 58). There were corresponding declines in population levels for the scenarios that included urban growth (Culvert 1, Culvert 3, and Open Culverts). Declines were especially notable in the Pahrump West zone, where most urban development is predicted to occur (Figure 59). The Open Culverts scenario in Trout Canyon also demonstrated significant losses, but this was likely due to stochastic processes inherent in the simulation. The area northwest of the city of Pahrump, while projected to have little additional disturbance, was predicted to have lower desert tortoise population sizes.

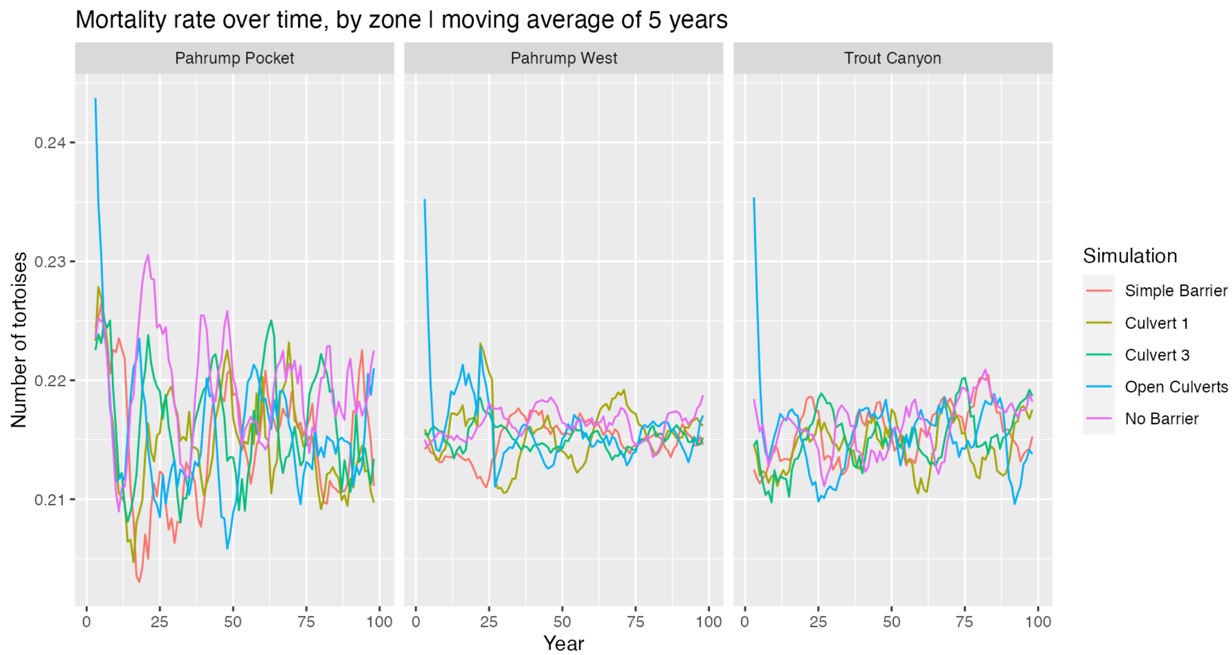

Figure 58 - Adult mortality rates in the Pahrump study area. Mortality proportions for adult tortoises are shown over time in each zone.

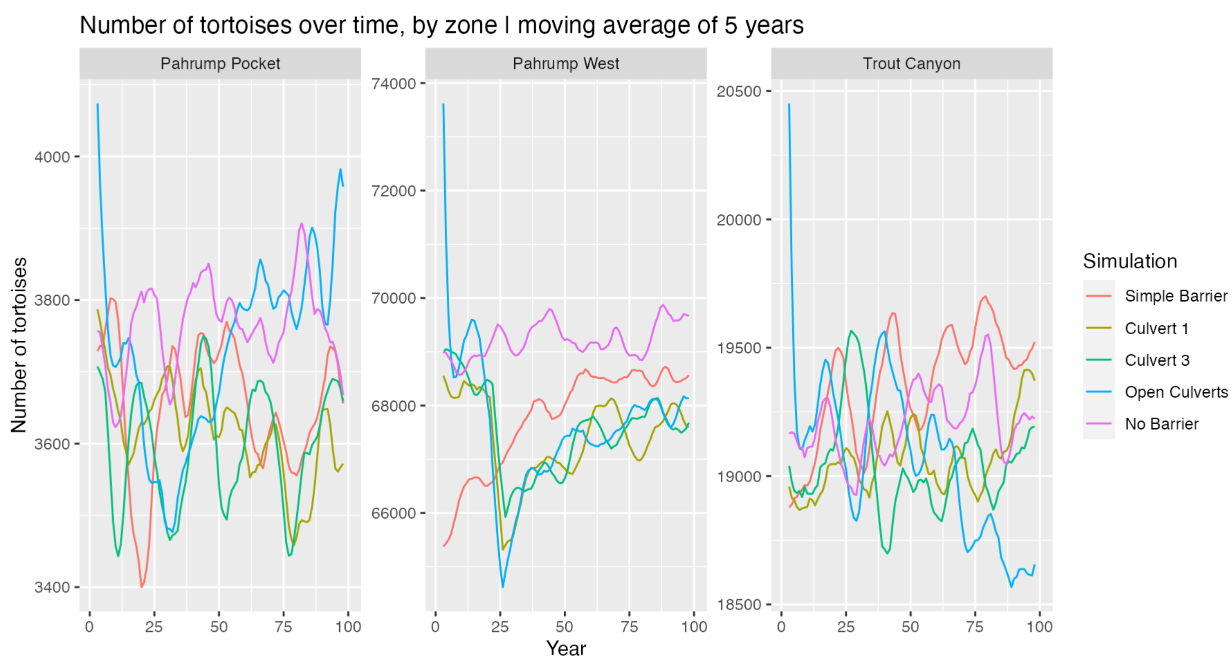

Figure 59 - Number of live tortoises in the Pahrump study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes.

Annual numbers of movements among zones are displayed in Figure 60, where the Simple Barrier and No Barrier maps indicate the minimal and maximum movement potentials between zones. The Culvert 1, Culvert 3, and Open Culverts scenarios showed similar numbers of animals predicted to cross zones through time. It should be noted in these scenarios that there is predicted to be little movement across the highway (US-372), as the only culverts noted on this roadway were at the extreme west end of the study area (Figure 60). There are also larger urban influences and dry lakes that provide areas of lower habitat along the highway. There was substantial exchange predicted between Trout Canyon and the Pahrump Pocket in the northwest (Figure 60). Movement between zones was much lower in the disturbance scenarios than in the No Barrier simulation, indicating again that the roadways as constructed are predicted to significantly limit movement, despite the presence of culverts, even if they are all equally passable (Figures 60 and 61).

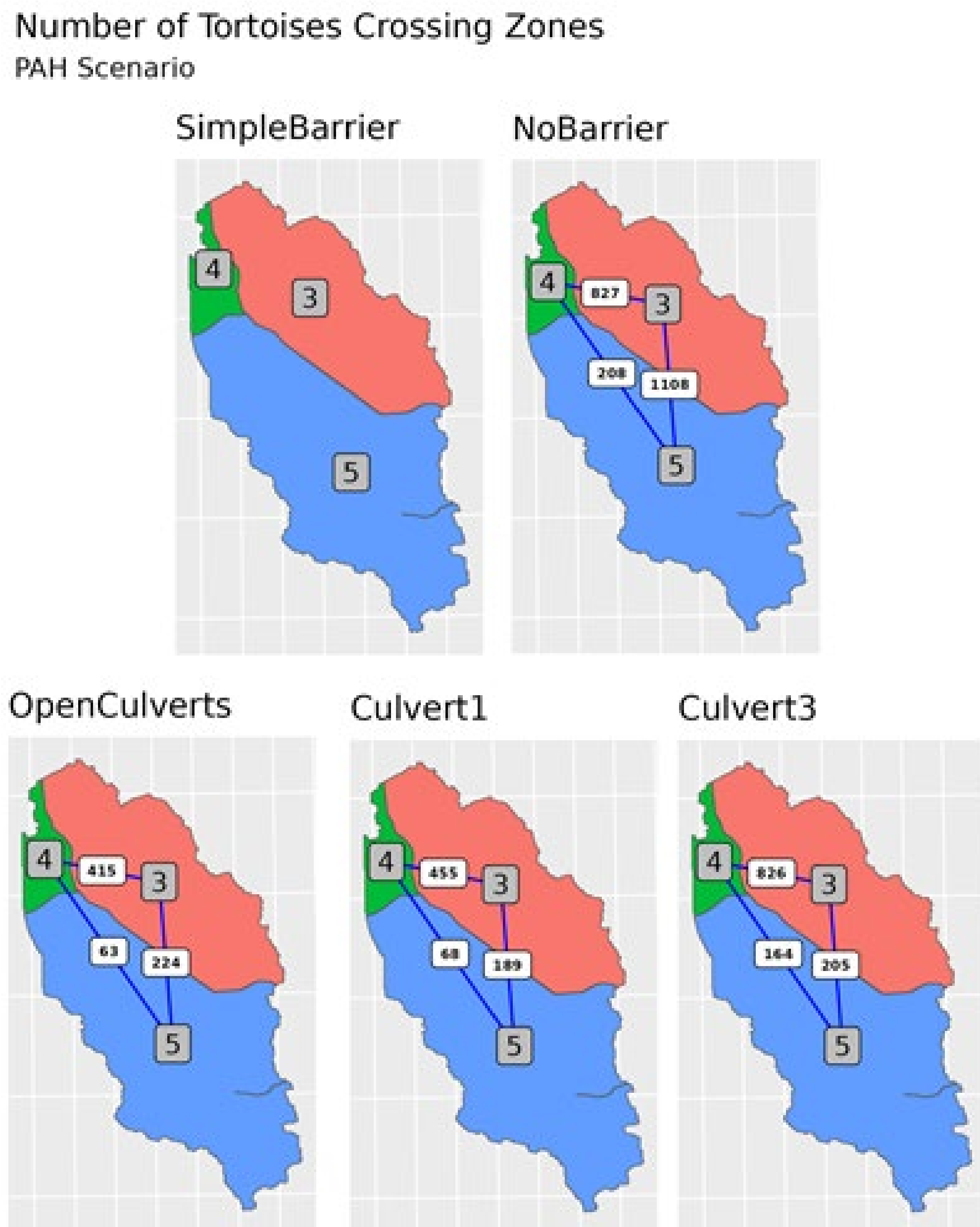

Figure 60 - The number of tortoises in the Pahrump study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels, and zone names are given in Table 9.

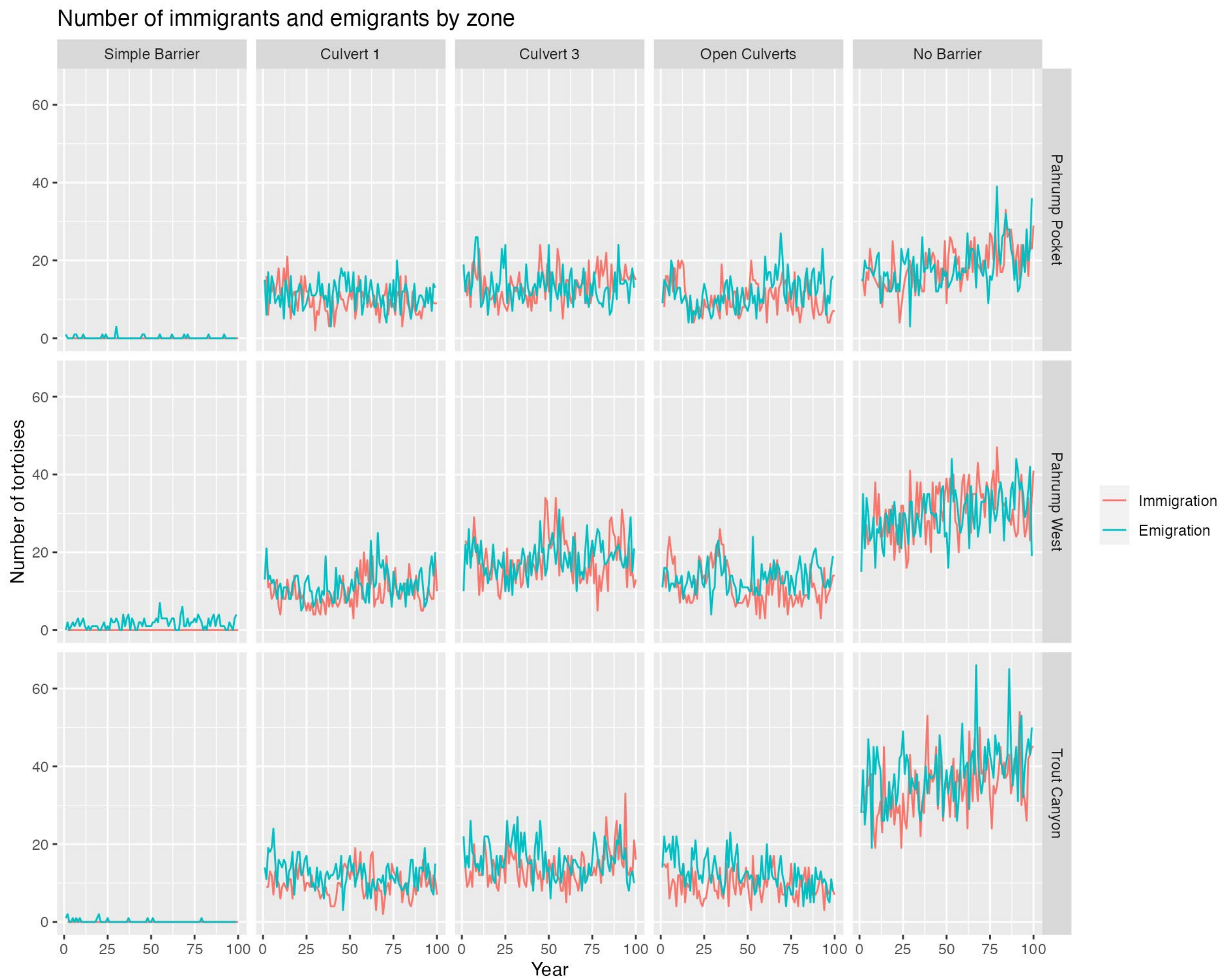

Figure 61 - Desert tortoise immigration and emigration in the Pahrump study area. Immigration and emigration are shown over time by zone for each scenario.

Mating by tortoises across zones, which would represent the basis for gene flow, was predicted to be much lower in disturbance scenarios than in a No Barrier simulation, as expected (Figure 62). In terms of disturbance scenarios, Simple Barrier showed little to no connectivity between zones, followed by Culvert 3, while Open Culverts and Culvert 1 performed comparably and were closest to the No Barrier simulation (Figure 62). Mating across zones was low between Pahrump Pocket and Pahrump West, regardless of scenario, and the most variable between Trout Canon to Pahrump West, with clear indications that Culvert 1 improved connectivity compared with Culvert 3 (Figure 63). The demographic summary plot showed increasing mortality associated with loss of habitat quality (Figure 64)

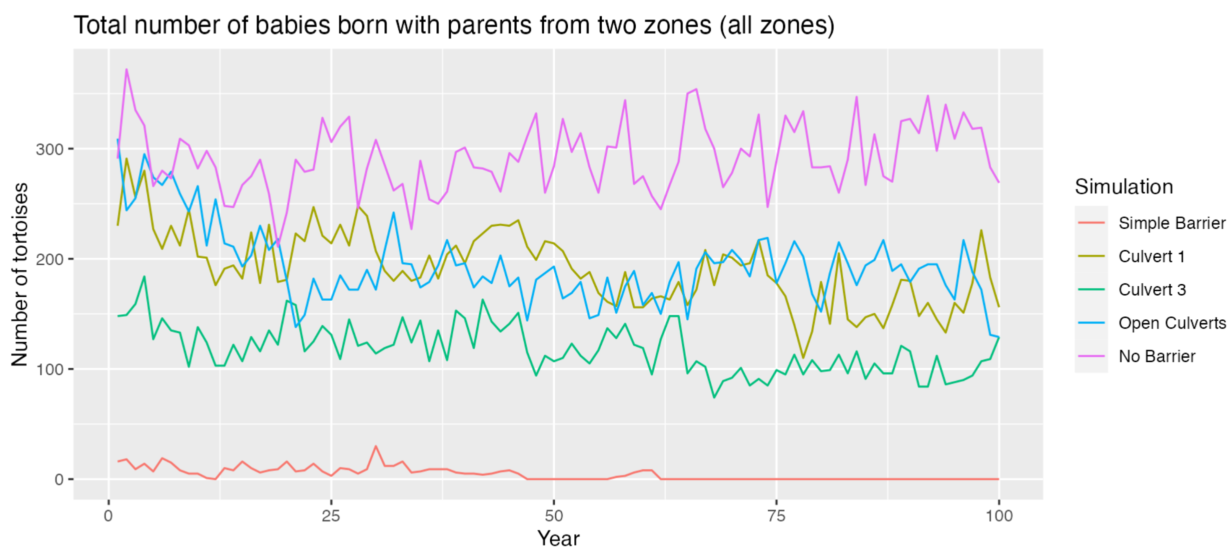

Figure 62 - Number of desert tortoises mating in the Pahrump study area. Mating is averaged across zones over time for each scenario.

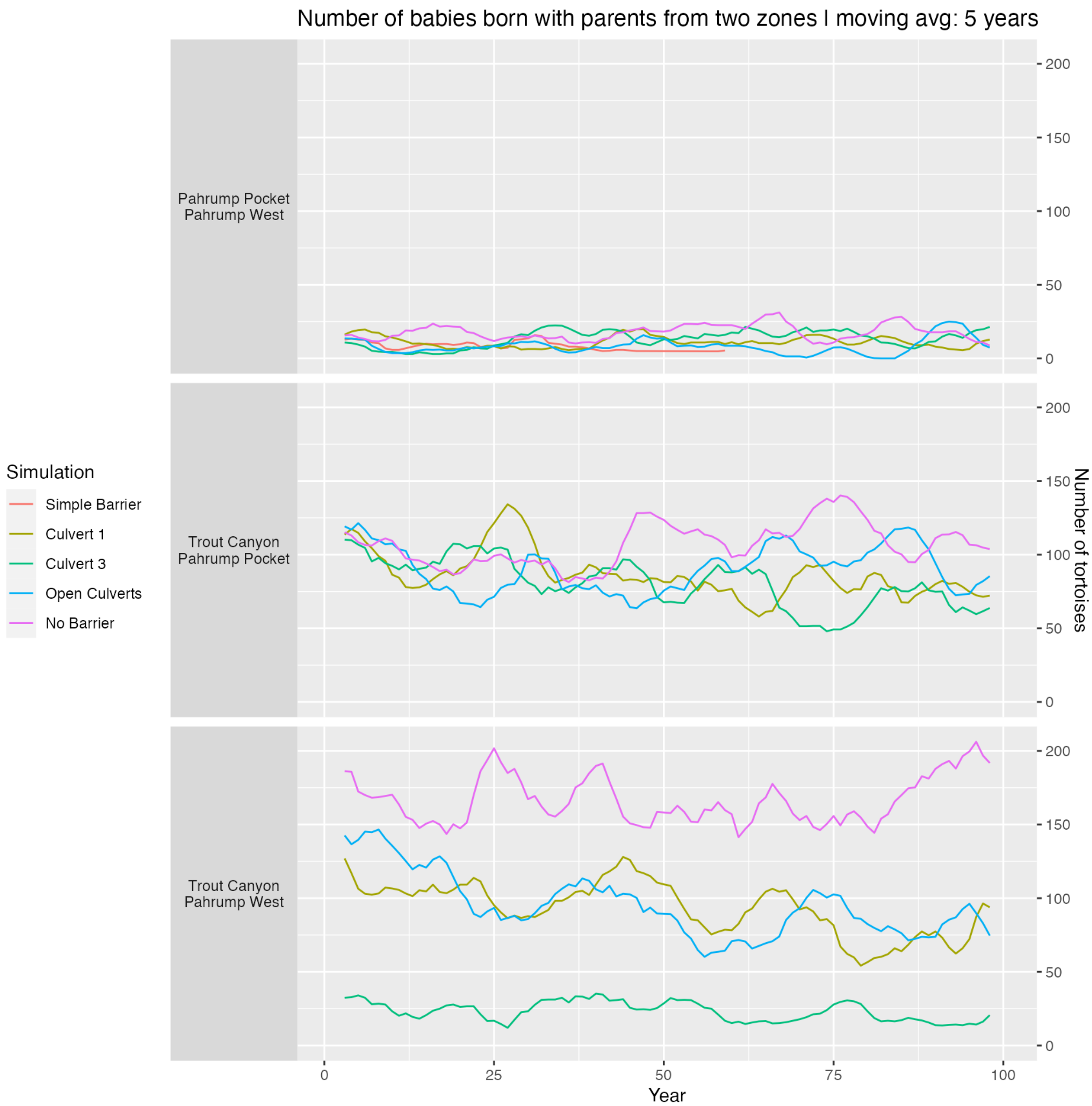

Figure 63 - Pahrump study area moving average of the number of offspring with parents originating in adjacent zones. Average values are displayed over time by zone for each scenario.

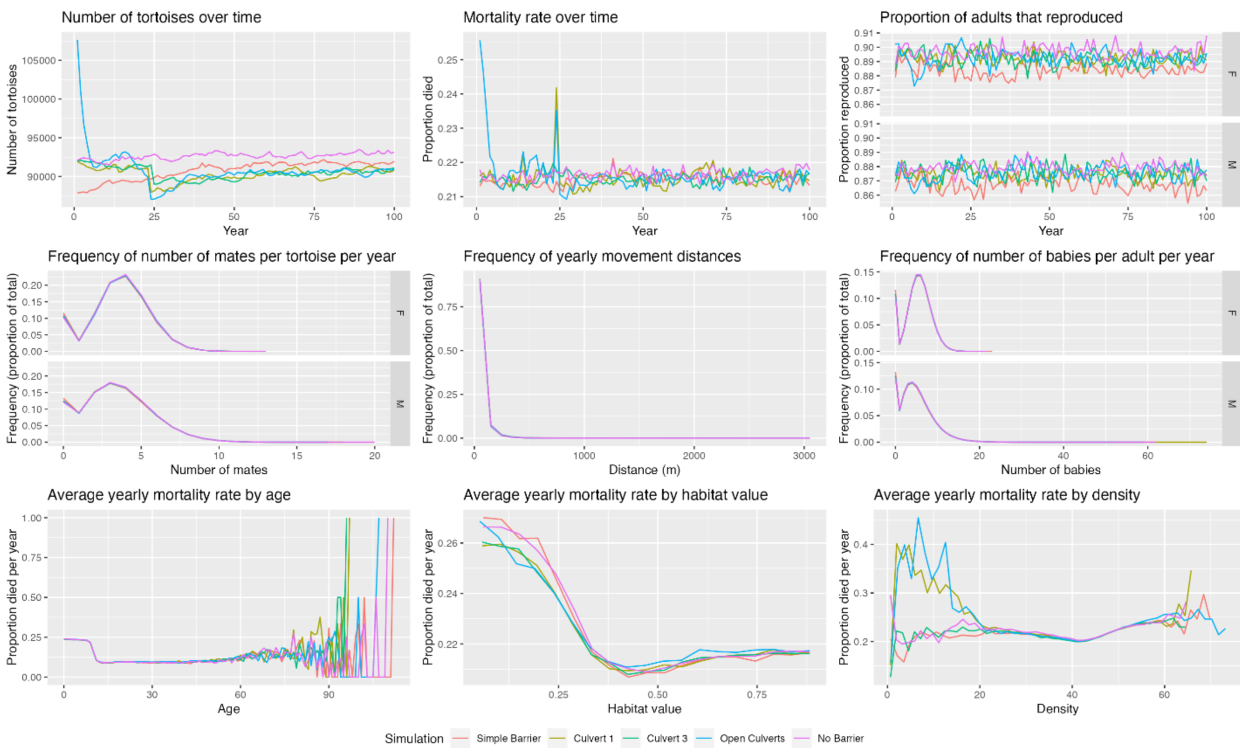

Figure 64 - Pahrump study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Highly significant pairwise F_{ST} values were noted between all zones in the Simple Barrier scenario, possibly indicating increased isolation when roadways lack culvert connections (see Supplemental Genetics Appendix for F_{ST} tables). Spatial structure analysis (sPCA) indicated a genetic cline in all scenarios, with the possible emergence of a genetic cluster in the northwest section of the landscape in the Simple Barrier scenario (Figure 65).

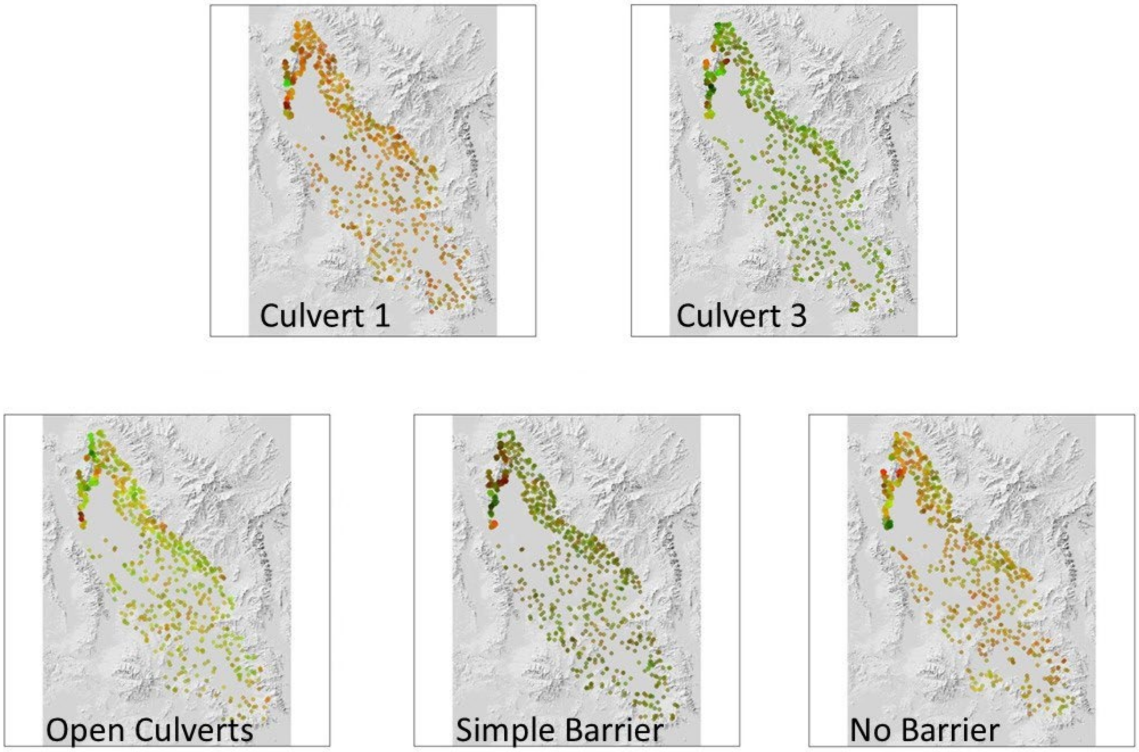

Figure 65 - Pahrump study area sPCA plots for each scenario. Points represent spatial locations; colors indicate assignment to genetic cluster. Genetic clustering appears to become more pronounced in the northwest corner of the Simple Barrier scenario.

Key takeaways from Pahrump area simulations

Tortoises in the barrier simulations showed losses of population numbers in response to predicted growth due to urban and solar development in the area. Pahrump Pocket, the smaller zone in the northwest, was predicted to have low numbers of tortoises and was relatively isolated from Pahrump West. The Pahrump West zone showed increasing isolation from the Trout Canyon zone, possibly resulting in genetic isolation over time. Urban growth in the Pahrump West zone could increase isolation of these populations. These results could indicate that maintaining connected culverts as safe passageways for desert tortoises across linear barriers in this area may be beneficial for connectivity. Culvert scenario recommendations based on simulation results:

- The Open Culverts and Culvert 1 scenarios behaved comparably, and either one is recommended for the study area.
- The Open Culverts or Culvert 1 scenario is predicted to improve connectivity along SR-160.

Landscape: Piute-Eldorado Valley

Piute-Eldorado Valley borders Nevada, California, and Arizona, and extends from Boulder City, Nevada south of Las Vegas to Needles, California. There is extensive solar energy development in the northern part of the study area, much of which is located within the town limits of Boulder City, Nevada. The valley is impacted by mining, roads, a new interstate highway (I-11), OHV use, transmission line rights of way, and utility scale solar facilities (Figure 66).

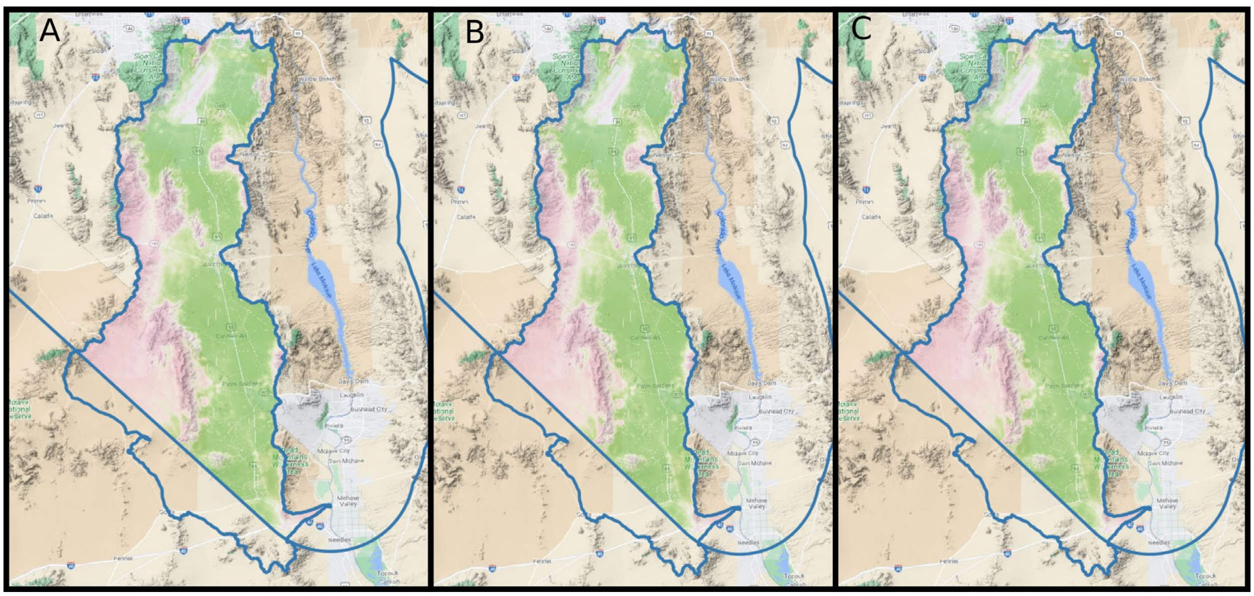

Figure 66 - Piute-Eldorado Valley study area showing habitat (green) with degradation due to roads, solar facilities, urban areas, railroads, and urbanization for years A) 2020, B) 2050, and C) 2100.

The region was divided into five primary zones for analysis (Figure 67 and Table 11). These depict areas that are separated by I-11, US-95, as well as smaller roadways - i.e. Nelson Road (SR-165), Nipton Road (SR-164, aka the Joshua Tree Highway), and the Laughlin Highway (SR-163). By and large the zones that were heaviest hit by development were Wee Thump North and BCCE North, as they have been subject to solar development and major roadways. Development throughout the southern portion of the study area was limited. Snapshots of area and habitat quality at the beginning, middle, and end of the simulations showed reductions in effective area in only one of the major zones because urban development removed habitat area. Changes in the average habitat quality (Table 12) were too small to be measurable. Habitat area in Wee Thump North (which was the only zone predicted to be impacted by urbanization) was reduced by roughly 1%.

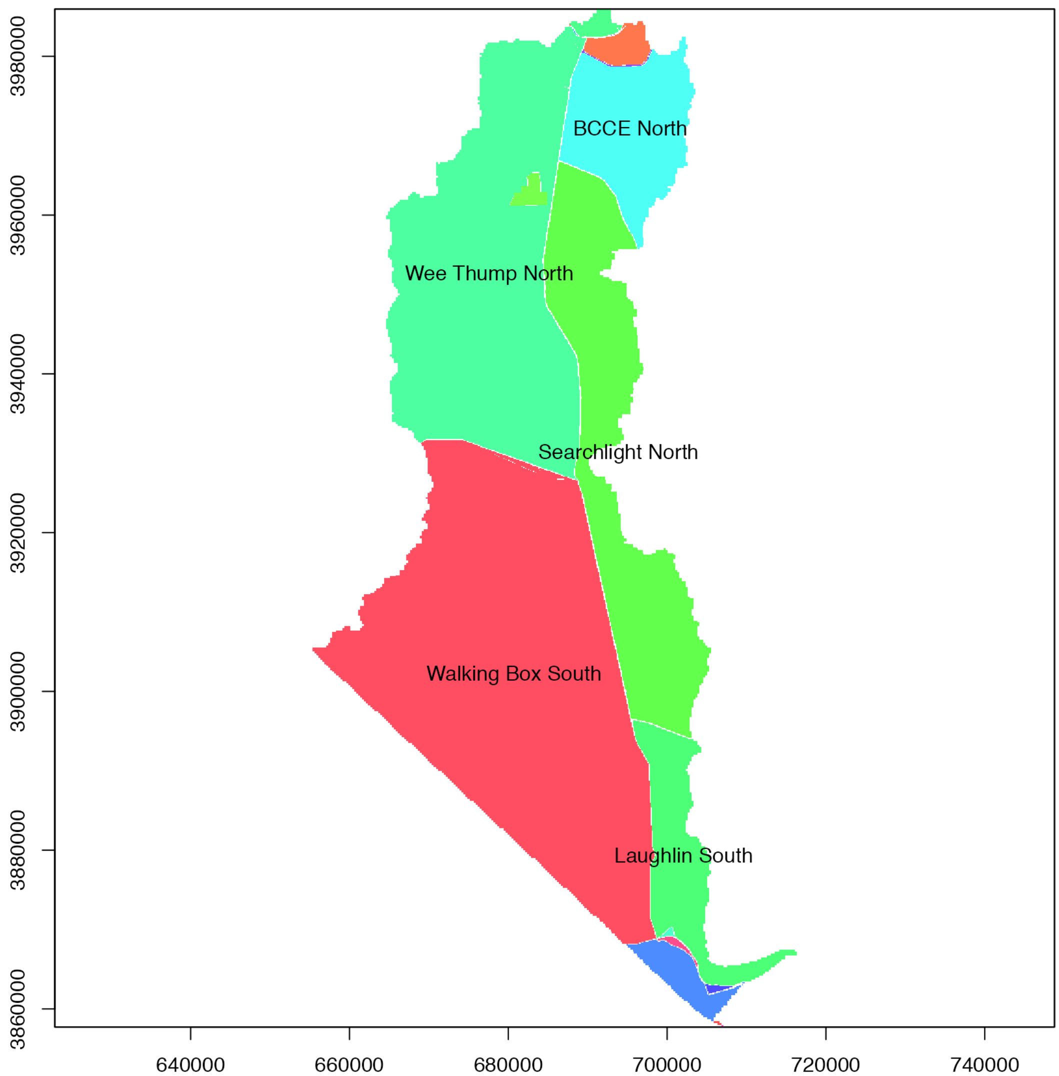

Figure 67 - Zones in the Piute-Eldorado Valley study area that were used for analyses.

Table 11 - Piute-Eldorado Valley zones. Larger areas separated by prominent boundaries within the Piute-Eldorado Valley area.

Zone	Zone Name	Description
18	Wee Thump North	West of US-95, north of Nipton Road
14	BCCE North	East of US-95, north of SR-165
26	Searchlight North	East of US-95, south of SR-165
48	Walking Box South	West of US-95, south of Nipton Road
39	Laughlin South	East of US-95, south of Laughlin

Table 12 - Piute-Eldorado Valley zonal changes. Zonal statistics showing changes in area and average cost value over time.

Zone Name	Area (km^2)				Mean Cost			
	2020	**2050**	**2100**	**Loss**	**2020**	**2050**	**2100**	**Change**
Wee Thump North	900.15	892.9	886.9	13.29	0.38	0.38	0.38	0.00
BCCE North	257.9	257.9	257.8	0.019	0.20	0.20	0.20	0.00
Searchlight North	548.69	548.7	548.7	0	0.20	0.20	0.20	0.00
Walking Box South	1421.0	1421	1421	0	0.39	0.39	0.39	0.00
Laughlin South	193.22	193.2	193.2	0	0.24	0.24	0.24	0.00

Demographics

Changes in demographics over time were minimal, but detectable in some zones. Adult death rates largely remained relatively constant, but were more variable in BCCE North and Laughlin South, with BCCE North showing a potential increase in mortality over time (Figure 68). Population levels were similarly variable, but remained at fairly high and constant levels, varying by a few hundred animals (Figure 69). Differences were not seen among zones, although the Open Culverts scenario appeared to have lower numbers for the BCCE North and Laughlin South zones, which coincides with the lowest predicted population levels.

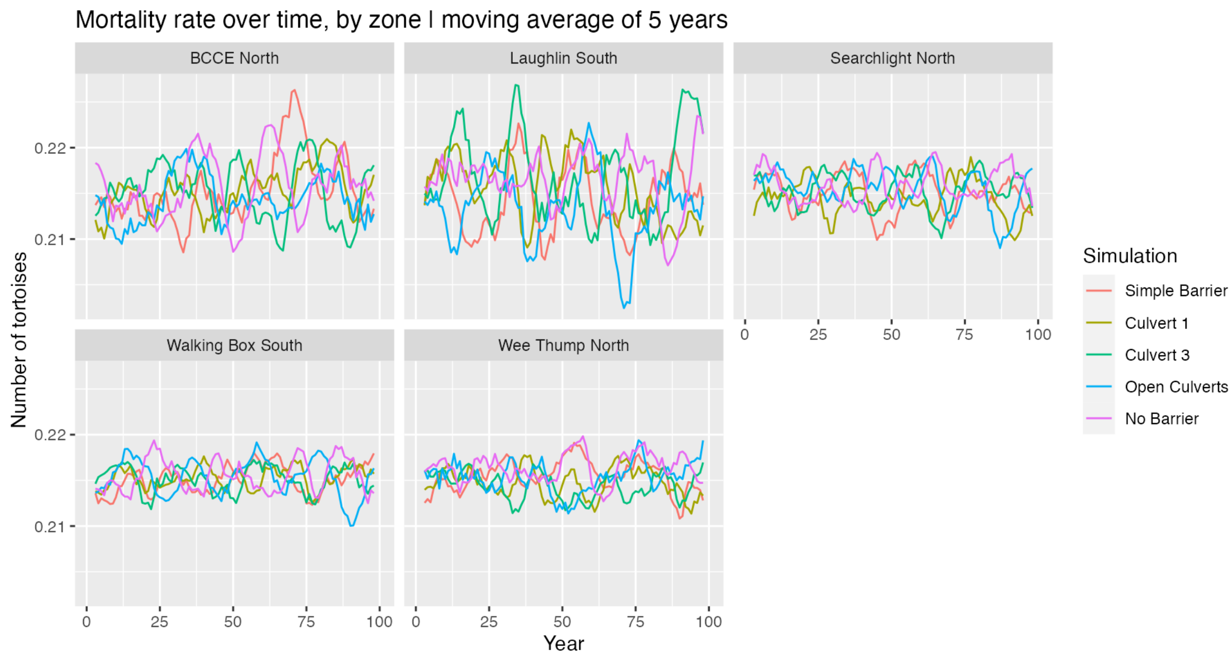

Figure 68 - Adult mortality rates in the Piute-Eldorado Valley study area. Mortality proportions for adult tortoises are shown over time in each zone.

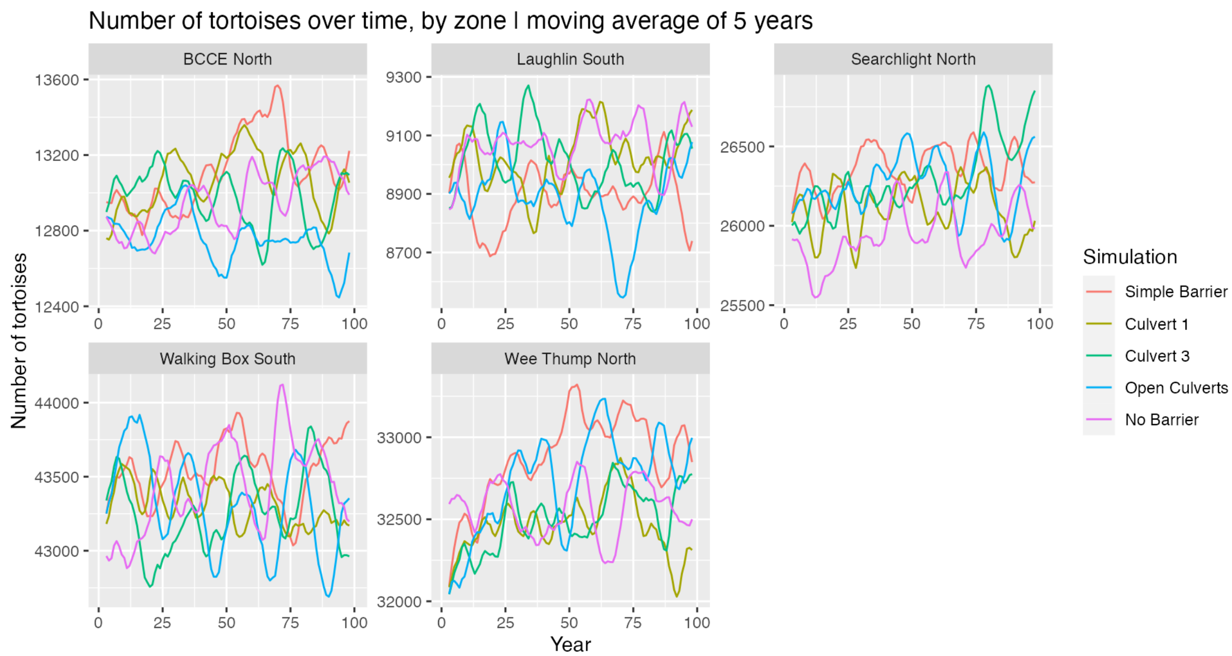

Figure 69 - Number of live tortoises in the Piute-Eldorado Valley study area. Live animals are graphed over time for each zone and by each scenario. Note differences in scale on y-axes

Numbers of movements among zones are displayed in Figure 70, where the Simple Barrier and No Barrier maps indicated the minimal and maximum movement potentials between zones. The culverts scenarios showed similar numbers of animals predicted to cross zones through time, although the number of tortoises that moved in and out of Searchlight North appeared to be higher in the Open Culverts scenario (Figure 70). The Culvert 3 (current condition) scenario showed reduced connectivity across the highway to Laughlin/Bullhead City between Searchlight North and Laughlin South (Figure 70). The most connected zones were Searchlight North to Wee Thump North, Walking Box South to Laughlin South, and Walking Box South to Searchlight North. However, these numbers were typically relatively similar to one another, with the exception of tortoises that moved between Searchlight North to Weethump South, which were highest in the Open Culverts scenario (Figures 70 and 71).

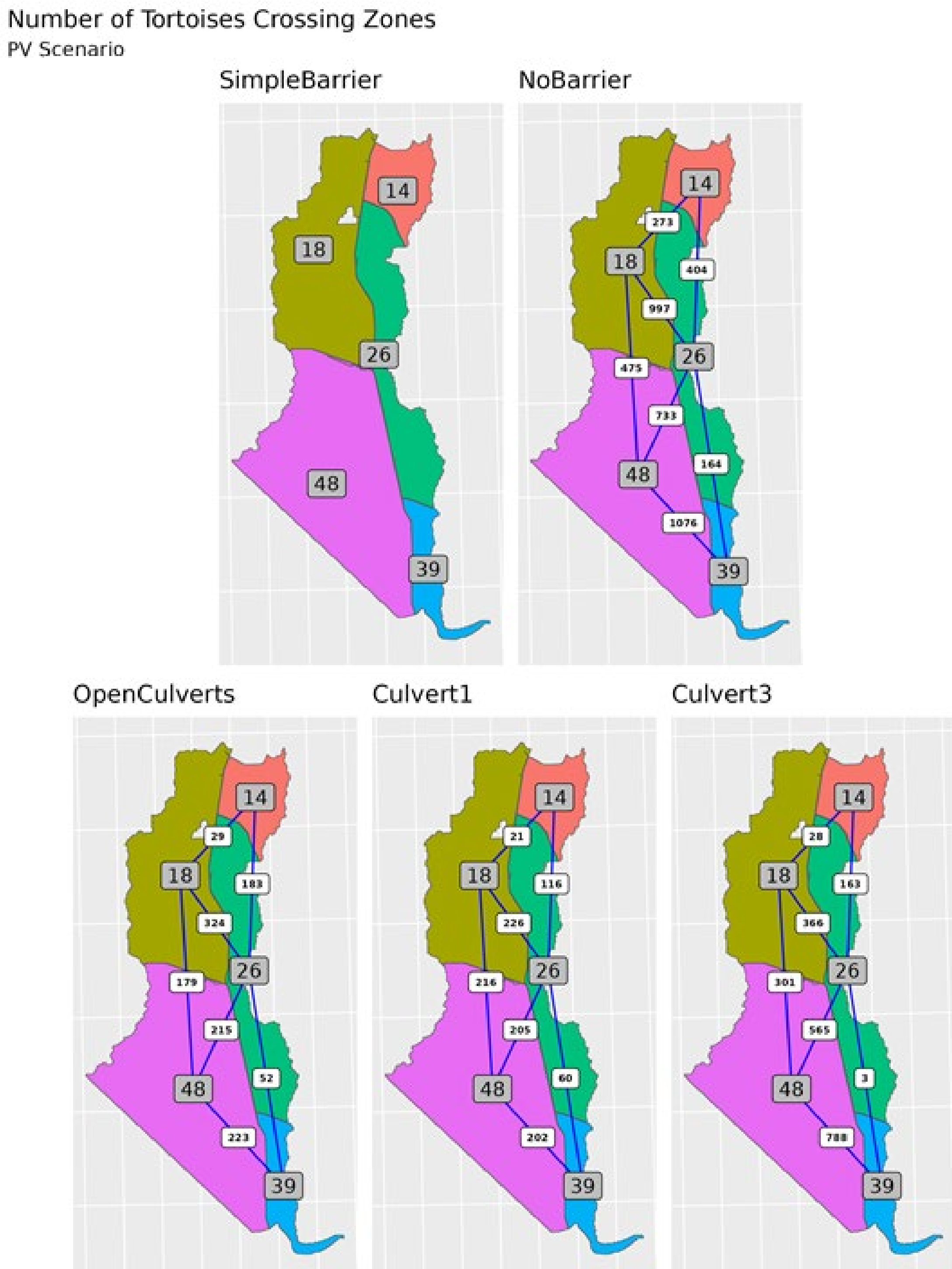

Figure 70 - The number of tortoises in the Piute-Eldorado Valley study area that moved between zones among years. White labels on lines indicate cumulative numbers of movements between zones. Zone numbers are indicated in gray labels; zone names are given in Table 11.

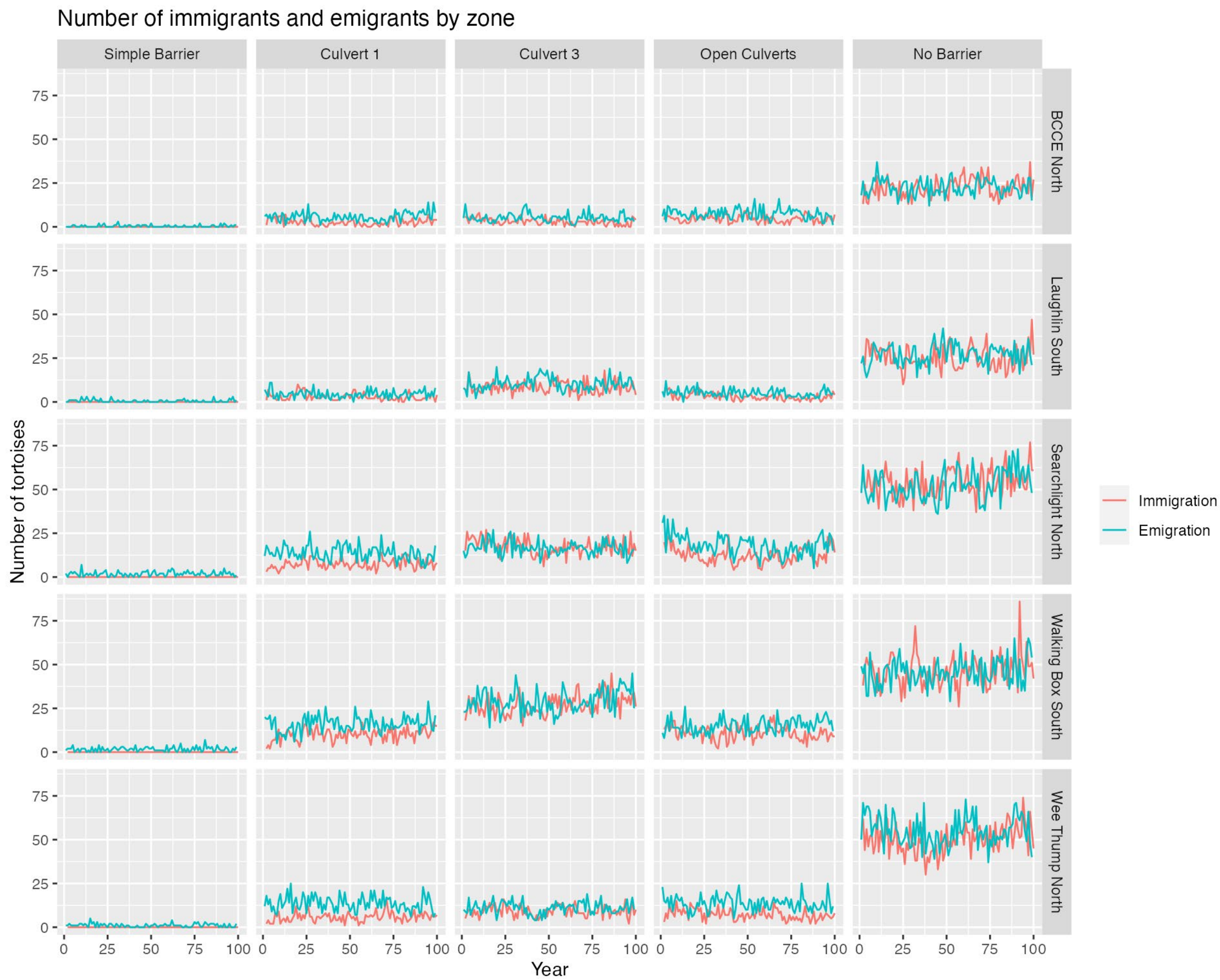

Figure 71 - Desert tortoise immigration and emigration in the Piute-Eldorado Valley study area. Immigration and emigration are shown over time by zone for each scenario.

Mating by tortoises across zones was lowest in disturbance scenarios and between Culvert 1 and Culvert 3, was lower in Culvert 3 (Figure 72). Over time reproduction between the zones appeared relatively stable in pairwise comparisons, with the lowest levels of connectivity predicted between BCCE North and Wee Thump North, Wee Thump North and Walking Box South, and Searchlight North and Laughlin South (Figure 73). Connectivity between Wee Thump North and Searchlight North was variable, but Culvert 1 outperformed Culvert 3 in our simulations (Figure 73). The demographic summary plot showed that as habitat values fell, so did population size (Figure 74).

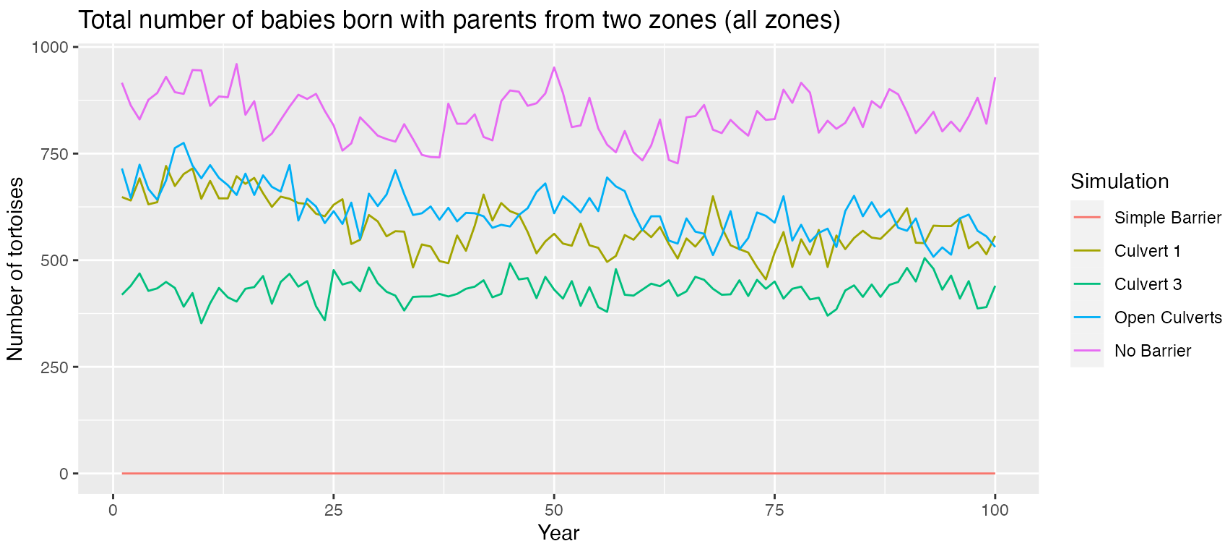

Figure 72 - Number of desert tortoises mating in the Piute-Eldorado Valley study area. Mating is averaged across zones over time for each scenario.

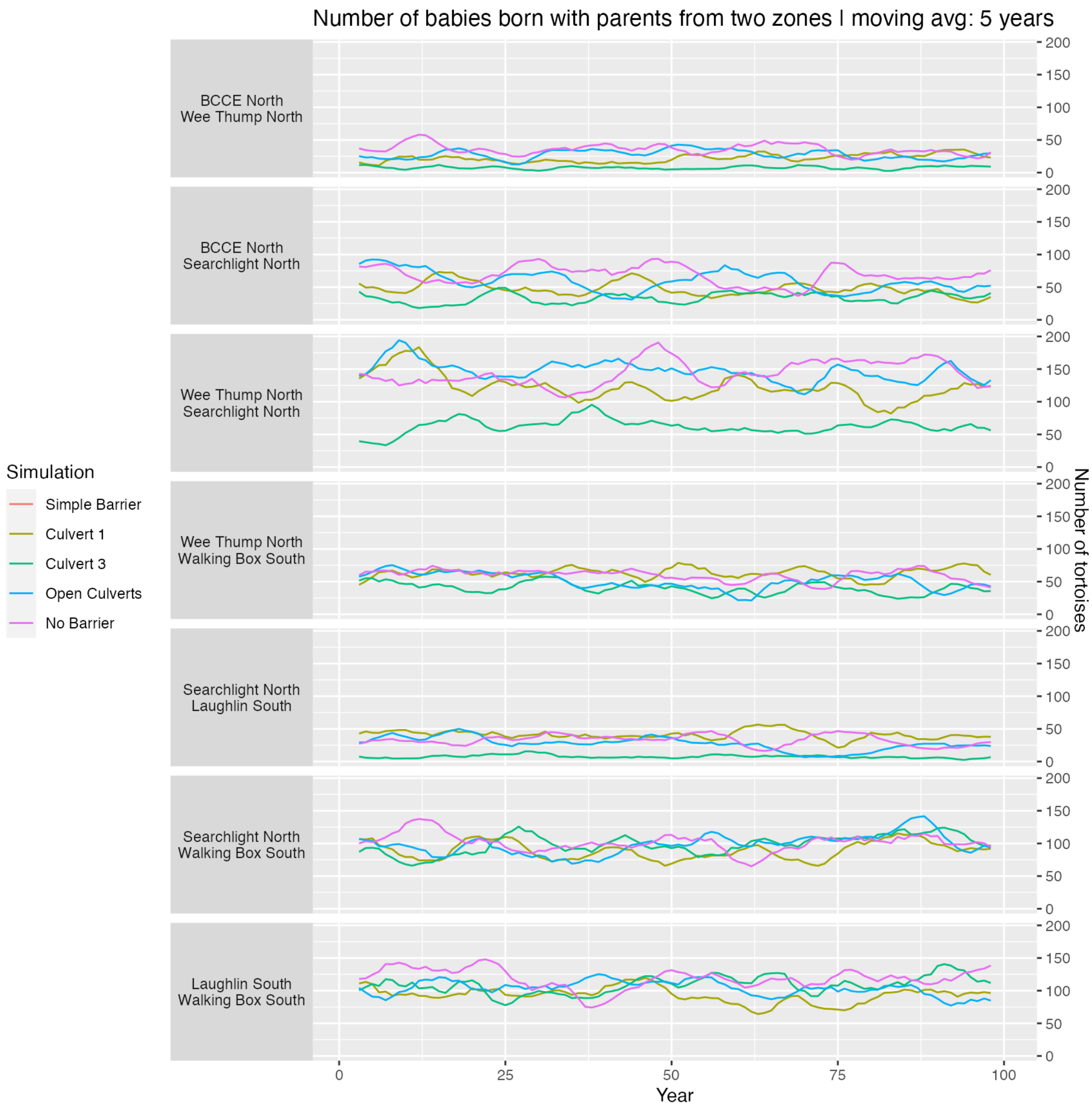

Figure 73 - Piute-Eldorado Valley study area moving average of the number of offspring with parents originating in adjacent zones. Average values are displayed over time by zone for each scenario.

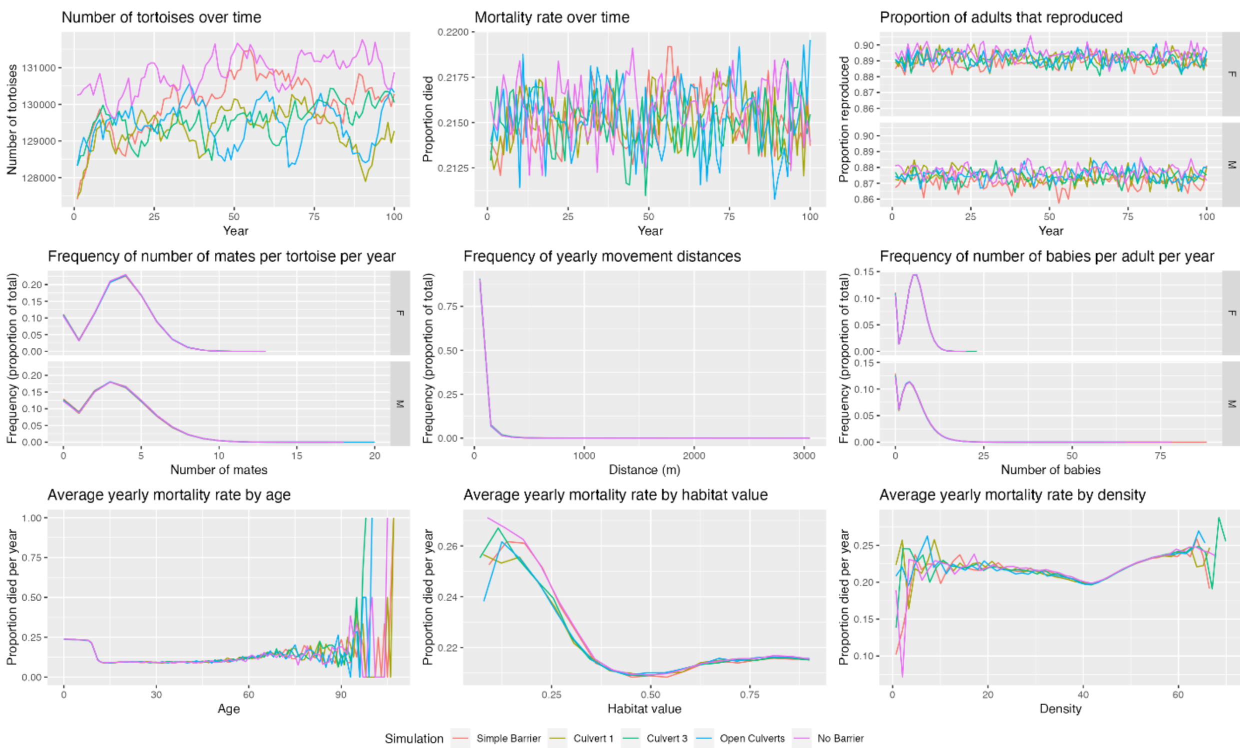

Figure 74 - Piute-Eldorado Valley study area demographic summary plot. The top row of plots depicts overall number of tortoises, mortality rates, and proportion of reproducing adults over time. The middle row shows yearly frequencies for number of mates, movement distances, and number of offspring. The bottom row displays average yearly mortality rates by age, habitat value, and density.

Genetics

Genetic differentiation between zones appeared relatively comparable by scenario (see Supplemental Genetics Appendix for F_{ST} tables); however, Culvert 1 and Culvert 3 indicated that values may increase most notable in the BCCE North zone, possibly as the result of the combination of less passable crossing structures and habitat disturbance (Figure 75). Allelic richness did not appear to be influenced by scenario. Heterozygosity was found to be significantly different between zones in the Open, Culvert 3, and Simple Barrier scenarios (*p*-value < 0.05). However, by zone, it appeared that BCCE North values trended lower in Culvert 1 and Culvert 3 than in other scenarios (Figure 76).

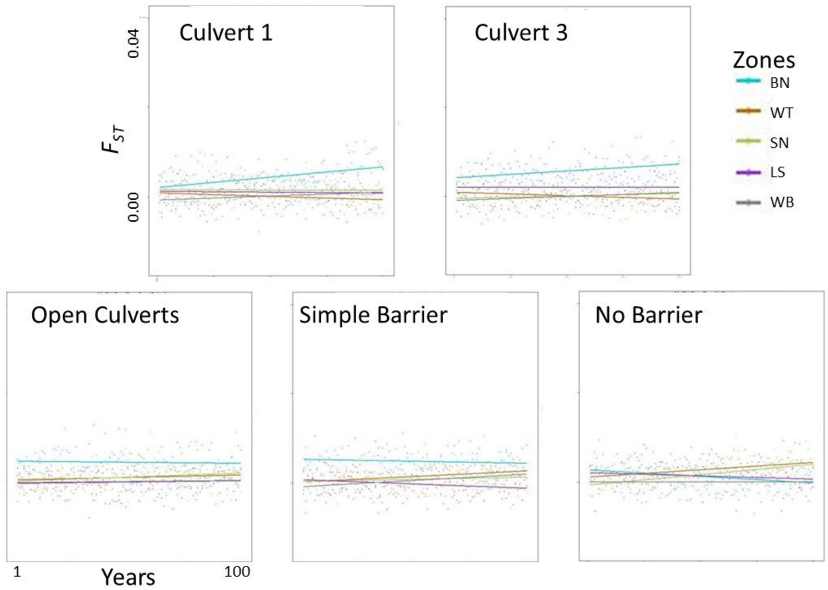

Figure 75 - Piute-Eldorado Valley study area genetic differentiation (F_{ST}) over time by zone for each scenario. Zones are BCCE North (BN), Wee Thump North (WT), Searchlight North (SN), Laughlin South (LS), and Walking Box South (WB).

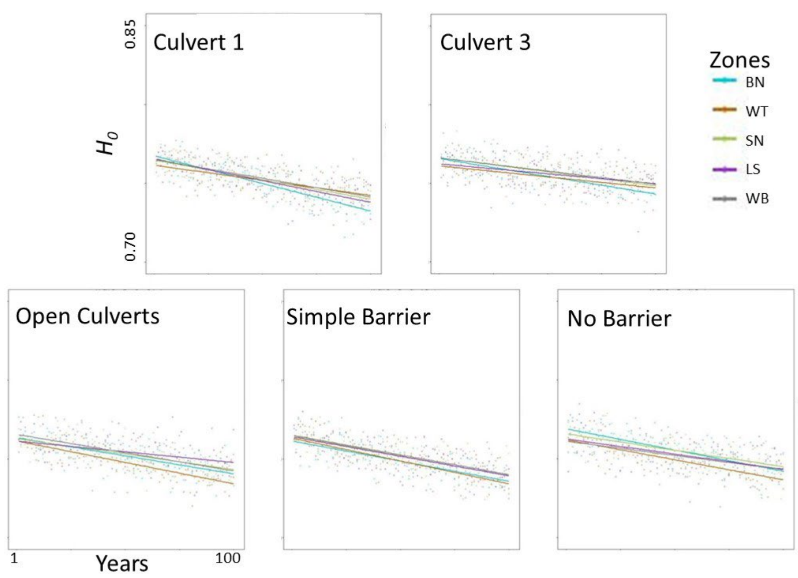

Figure 76 - Piute-Eldorado Valley study area heterozygosity (H_o) over time by zone for each scenario. Zones are BCCE North (BN), Wee Thump North (WT), Searchlight North (SN), Laughlin South (LS), and Walking Box South (WB).

Key takeaways from Piute-Eldorado Valley simulations

Tortoises in the barrier simulations were predicted to show similar demographic patterns. Due to habitat arrangement, some zones had higher population levels than others, and those with smaller population sizes tended to have the appearance of higher variability in annual population sizes and mortality rates. Where urban growth was predicted to be minimal it did not result in a substantial predicted losses of animals. Movement was relatively consistent between culvert scenarios, with significant reductions (e.g. by 75%) relative to the No Barrier scenario. Thus, existing roadways and barriers served to reduce connectivity among these populations. Taken together, this could indicate that maintaining passable culverts in light of minimal predicted development in the northern portion of Piute-Eldorado Valley may be most beneficial for connectivity. Culvert scenario recommendations based on simulation results:

- Either the Open Culverts or Culvert 1 scenario is predicted to best maintain connectivity throughout the study area.
- Specifically, the Open Culverts or Culvert 1 scenarios are recommended along US-95 and Nipton Road, and highway 163 to Laughlin.

DISCUSSION

As expected, the barriers in each of the landscape scenarios reduced connectivity, with the loss of connectivity predicted to be mediated to some degree by culverts. Culverts are widely used by wildlife, and can provide benefits for connectivity across roadways for many species (Taylor and Goldingay 2003; Mata et al. 2003); therefore, estimation of their effectiveness is of key importance (Seiler et al. 2016). We were able to evaluate the relative impacts of roadways by bounding the expectation of both full and severely limited connectivity by modeling scenarios with no barrier represented at all, barriers with culverts accessible to varying degrees and urbanization, and closed culverts representing absolute barriers (Simple Barrier). We also modeled reductions in connectivity that differed by road type and habitat configuration, ranging from 0 to 100% reduction, but overall road barriers had an average reduction of 62% (see collective average movement graphs throughout). These losses in connectivity typically resulted in a reduction in the number of offspring with cross-zonal parents, but did not appear to otherwise consistently affect other demographic parameters. For example, each of the zones that had lower numbers of animals crossing between adjacent zones in the Northwest corridor study area (Figures 50 and 51) also had fewer offspring with cross-zonal parents (Figure 53), contributing to an overall reduction in connectivity effect on offspring (Figure 54). Losses of individuals were possible due to elevated risk on roadways, but in our modeling effort fenced roadways did not allow for animals to cross. It was also the case that we did not consider aversion to roadways in our simulations, and this has been noted in desert tortoises and other chelonians (Nafus et al. 2013; Shepard et al. 2008), and could further reduce connectivity (Seiler et al. 2016).

Urban growth also resulted in losses of habitat and impacted tortoise population numbers in several of the study areas. Three zones in the Ivanpah Valley lost 13 to 20% of tortoise habitat, and had losses of up to 21% of animals in those zones (Figures 13 and 14). Several zones in the Northwest corridor were predicted to lose from 9 to 18% of habitat across the 100-year simulation period, with sustained losses of up to 15% of individuals (Figures 47 and 48). In addition, the Hayford zone in the North area lost 5% of habitat and showed reduced population sizes over time (Figure 36). This same area was subjected to large losses of habitat in the 2005 wildfires that swept through the area (Drake et al. 2015), and consequently tortoises may be at lower densities than modeled here. Tortoise populations have declined by roughly one-third in a recent decade (2004 to 2014) with recommendations to more critically evaluate development and human activities in tortoise habitat (Allison and McLuckie 2018). Our results show support for continued declines in tortoise populations with increased urbanization.

This modeling effort demonstrates the potential impacts of urbanization and major barriers to movement on desert tortoise populations throughout Clark County, Nevada. Based on these simulation models we anticipate that increased urbanization will not only reduce population levels, but that over time reduced connectivity will contribute to genetic isolation, especially when populations were predicted to be smaller and more fragmented. Culverts on roadways demonstrated improved maintenance of connectivity; however, the assumption that all culverts will be used by tortoises should be evaluated. Our simulations used high levels of anticipated human population growth and represented a large urban growth expectation for the study region. Given historic trends in urban expansion in the area this is a reasonable expectation, but it does not represent all human population growth scenarios. Should urban growth trends slow over time, or increase more dramatically than predicted, the realized effects on desert tortoise demography and connectivity may differ from results presented here. Additionally, our simulations were run one time for each scenario. Due to the variability inherent in simulation modeling, especially when population sizes are low, our results may not adequately account for stochasticity. Ideally, each simulation scenario should be run repeatedly and averaged; however, due to computation times and intensity replicate runs were not possible for this effort.

Genetic metrics did not show strong responses over the 100-year simulation period, which is a different outcome than the results found by Dutcher et al. (2019). There are several likely causes for these differences. First, our simulations included overlapping generations in annual simulations over a 100-year period, while Dutcher et al. modeled sequential generations 17 years long for 200 generations, equating to 3,400 years. Second, the impacts due to habitat change often occurred later in our simulations, limiting the time over which any effects could manifest in a genetic signal, as these effects likely take many generations to be detectable (Cushman et al. 2006; Holderegger and Giulio 2010; Dutcher et al. 2020).

Urban growth in our simulations impacted our study areas, and the zones within our study areas during different time periods (Figure 77). The higher areas of urban growth over time were first realized in the Ivanpah Valley, along the Nevada-California border, and were associated with the build-out of the airport near Jean, Nevada. Other areas of high growth were seen in the Northwest corridor and in the North area, and were largely expansions of the already urbanized city in the greater Las Vegas Valley. These spatial and temporal differences in urbanization differentially affected tortoise populations (as described above). The impacts can be readily seen in the demographic profiles (e.g. periods of predicted higher mortality rates followed by sustained reductions in population size in many areas). The gradual progression of urban grown resulted in corresponding changes across the 100-year simulation, and given that genetic changes take longer in to detect in long lived species with overlapping generations (Holderegger and Giulio 2010), we would expect more time is needed before we might see significantly measurable effects on genetic metrics in those populations (Cushman et al. 2006). Still, in areas that had smaller isolated populations we did see the indications of change in ways that are indicative of genetic isolation. Genetic differentiation has been found to gradually increase with time and increased fragmentation (Segelbacher et al. 2003). Over time we predict tortoise populations with indications of isolation would show stronger signals of fragmentation and negative genetic effects, such as loss of genetic diversity.

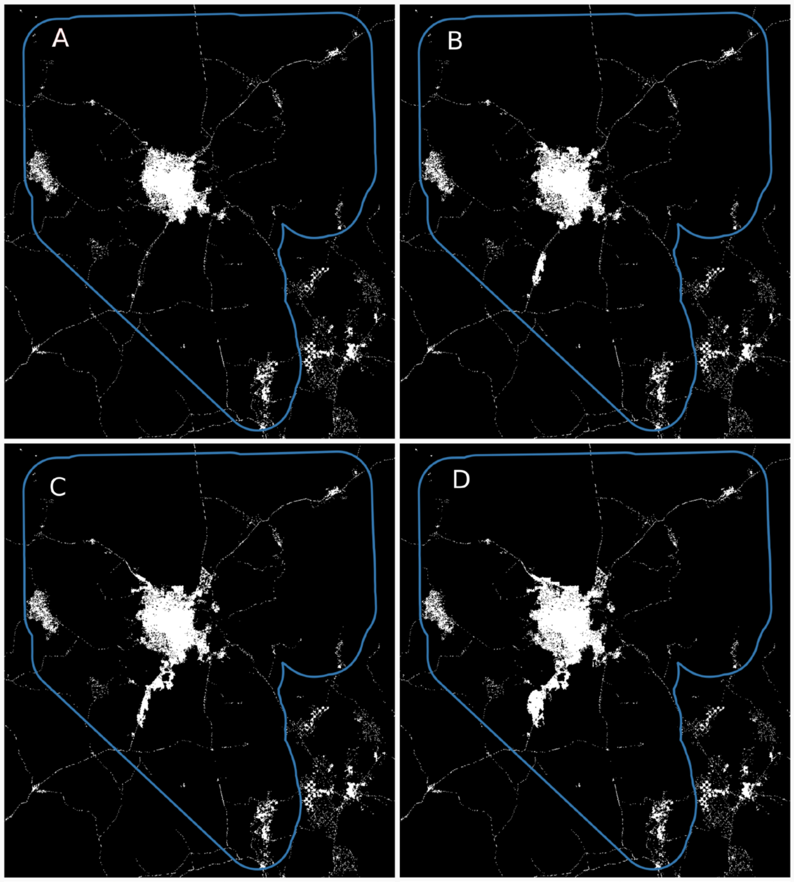

Figure 77 - Snapshots of urban growth over time within Clark County, Nevada for years A) 2025, B) 2050, C) 2075, and D) 2099.

Tortoises tend to occur in areas with little anthropogenic disturbance (< 5% within 1 km; Carter et al. 2020). We modeled reductions in habitat quality attributed to existing dirt roads and direct losses due to current and predicted future urbanization. However, we did not model the associated degradation of habitat that often occurs in and around these areas (Theobald et al. 1997; Liu et al. 2016). Tortoise abundance has been associated with diverse perennial vegetation communities, which are reduced by vehicular and human use (Berry et al. 2014). OHV use was restricted to existing trails and routes, but it is likely that over a 100-year time series, habitat adjacent to urban areas and OHV areas would degrade substantially over time (Theobald and Romme 2007), causing further reductions in tortoise habitat and populations.

This may impact tortoise movement patterns, as they have been found to avoid areas with low perennial vegetation cover and low density roads, but will travel along fences (Hromada et al. 2020). Because they will travel along fences, often quickly (Peaden et al. 2017), tortoise fencing tied in with passable culverts is likely to increase connectivity. Increased connectivity was seen in our simulations when more culverts were tied in relative to the current condition, even without the fence following behavior included in the movement algorithm. Recommendations to reduce adult mortality, such as limiting shooting areas and OHV access, have also been proposed (Doak et al. 1994).

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