Title: Assessing Changes in Clusters of Wildlife Road Mortalities after the Construction Wildlife Mitigation Structures

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Running Headline: Changes in Wildlife Road Mortality Clusters

Abstract

Collisions with vehicles are a major threat to wildlife populations and often occur in identifiable patterns. To reduce wildlife road mortalities, mitigation structures including exclusionary fencing and wildlife crossings are constructed. Openings in fencing at road intersections may lead to concentration of road mortality hot spots at openings leading to a belief that these gaps concentrate road mortalities. However, it is also possible that hot spots existed at these locations before construction indicating that road mortality patterns have not changed with mitigation structure construction. Therefore, to assess mitigation structure effectiveness, it is important to examine both road mortality numbers and road mortality spatial distribution. Wildlife road mortality data was collected on a 15-km section of rural highway in Texas, USA before, during, and after the construction of wildlife mitigation structures. We expected that the number of road mortalities would decrease after construction compared to before construction and that road mortalities would become more concentrated around openings in the fence. We used ANOVA to compare numbers of road mortalities and emerging hot spot analysis and generalized linear modelling to assess changes in road mortality spatial distribution. Road mortalities were not significantly different in the before and after construction periods ($p = 0.092$). While there were no significant changes in road mortality patterns with construction, cluster intensity was greater when nearer to fence openings in all three time periods. Emerging hot spot analysis provides an effective and easy way to visualize road mortality patterns through time, however, due to low numbers of mortalities in many road mortality studies, including this one, the power of this analysis to detect significant changes in road mortality may be limited. This technique can provide both ecologists and transportation planners an effective tool for identifying patterns that may warrant further investigation using traditional statistical techniques.

Keywords: emerging hot spot analysis; Mann-Kendall test; road ecology; wildlife road mortality; wildlife mitigation structures

1 Introduction

The distribution of wildlife road mortalities (WRMs) is not random and is often associated with surrounding habitat and access to the road (Clevenger, Chruszcz & Gunson 2001; Ascensão *et al.* 2017). Clustering of WRMs represents unique patterns of WRMs that may be associated with different environmental factors than WRM counts (Snow, Williams & Porter 2014; Bíl *et al.* 2019). In the road ecology literature, clustering is often mistaken as simply areas with a large number of WRMs (Teixeira *et al.* 2017), however these locations may not represent true clusters of WRMs and may lead to misinterpretation of which factors influence WRMs (Bíl *et al.* 2019). Misidentification of where clustering occurs could lead to wildlife mitigation structures being placed in areas where they may not be most needed (Andis, Huijser & Broberg 2017; Teixeira *et al.* 2017).

An effective examination of WRM clustering requires analyses that identify the statistical significance of an identified cluster. Statistics that exist to identify statistically significant clustering include hot spot analysis and Moran's I analysis (Caldas de Castro & Singer 2006; Grubesic, Wei & Murray 2014). Both methods can identify the exact locations of a statistically significant cluster using localized versions of the tests (Caldas de Castro & Singer 2006). When examining clustering in WRMs, identifying where clustering occurs is more important than determining that clustering occurs (van der Ree, Smith & Grilo 2015).

Local hot spot analysis measures how different a group of cells is compared to its surrounding cells by providing a measure of how concentrated those cells are (Getis & Ord 1992) while local Moran's I analysis identifies how an individual cell differs from its surrounding cells (Anselin 1995). These two methods can complement each other and provide a better overall assessment of where clustering occurs along a highway (Ord & Getis 1995). In addition, by performing these analyses at the same spatial scale in different time periods, one can assess how the intensity of a WRM cluster changes through time (Getis & Ord 1992).

Once WRM clusters have been identified, we can examine how environmental factors influence the intensity of said cluster and how this influence varies in time. Factors that influence the distribution of WRM clusters include variation in landcover and land use (Caceres 2011; Ascensão *et al.* 2017) and the properties of the highway (Clevenger, Chruszczc & Gunson 2003; Grilo, Ferreira & Revilla 2015). Wildlife mitigation structures, especially exclusionary fencing, may also influence the intensity of WRM clusters (Cserkész *et al.* 2013). Fencing restricts access to roadways to narrow gaps at road intersections and private drives which can decrease the overall number of WRMs on the highway (Forman *et al.* 2003), however it could increase the intensity of WRM clusters near these locations by funneling animals towards gaps in the fences (Cserkész *et al.* 2013). The potential for funneling is often a concern in wildlife mitigation structure construction (Huijser *et al.* 2016) so gaps are often mitigated by different structures including gates, wildlife guards, and wing walls. These structures are not 100% effective at keeping wildlife off roads and may still result (Allen, Huijser & Willey 2013; van der Ree, Smith & Grilo 2015). Examining how fence gaps influence the intensity of WRM clusters may be important in determining how wildlife mitigation structures affect WRMs.

We describe methods for identifying how WRM clusters change through time with the construction of wildlife mitigation structures on State Highway 100 (SH100) in Cameron County, Texas, USA. We examined how the intensity of WRM clusters changed with mitigation structure construction at a fine temporal scale and how factors influencing road mortality clusters change from before construction to after construction of wildlife mitigation structures. We expected the overall number of WRMs to decrease after construction, but the intensity of WRM clusters to increase after construction. We also expected that the intensity of WRM clusters will decrease with increased distance to wildlife mitigation structures in the after-construction period only.

2 Methods

2.1 Study Area

The study area was a 15-km section of SH100 in Cameron County, Texas, USA between the towns of Laguna Vista and Los Fresnos (Fig. 1). The highway was a four-lane divided road where the directions of traffic are separated by a concrete traffic barrier. This section of SH100 had a speed limit of 105 kmh and an average annual daily traffic of between 7000 and 9000 vehicles (Texas Department of Transportation 2019).

Wildlife mitigation structures were built along an 11.9 km section of the study area between Sep 2016 and May 2018 which included exclusionary fencing, five wildlife crossings, 18 wildlife guards, three wing walls, and 16 gates. The mitigation structures were designed to prevent ocelots (*Leopardus pardalis*), bobcats (*Lynx rufus*), and other medium to large mammals from accessing the road, while still providing connectivity across the highway (Environmental Affairs Division 2015). The fencing material was 5.1 cm wide black plastic-coated chain-link, 1.8 m tall, and along most of the fence line is buried 30.5 cm into the ground. In areas where burial was not possible, the fence was secured to the ground away from the highway.

2.2 Wildlife Road Mortality Methodology

Wildlife road mortality surveys were conducted before, during, and after the construction of the mitigation structures on SH100. The survey transects included the full mitigation area as well as a 1.5 km buffer on both sides. Survey frequency, speed, and marking differed (Table 1), resulting in differences in the total number of surveys conducted among periods. The total number of surveys were 30 before construction (Aug 2015 – Aug 2016), 127 during construction (Sep 2016 – May 2018), and 67 after construction (Jun 2018 – Sep 2019). The switch to one survey per week was due to a previous study on SH100 that showed that most carcasses lasted for at least a week (Livingston 2019).

Only those species for which fencing provided a barrier to movement were used in analyses to assess how fencing changed WRM patterns. These included all mammals larger than rodents as well as turtles and tortoises (Table 2). Snakes, amphibians, birds, and small mammals were not included in analyses; see Appendix A for a complete list of species found during WRM surveys.

2.3 Land Classification

To identify land use and land cover types around SH100, we created a classified vegetation map using the Interactive Supervised Classification Tool in ArcMap 10.6 (ESRI 2017). We identified 10 classes: trees, mixed trees/shrubs, cactus, cord grass, open, bare, paved road, dirt road, water, and bahia. Classification was confirmed by visual inspection of the map. These classes were reduced to three major land cover classes: forested (trees and bahia), shrub (mixed trees, cactus), and open (open, bare, paved road, and dirt road). The water class was excluded because water was identified using a different method, described below.

We identified permanent sources of fresh and salt water using the National Wetlands Inventory (U.S. Fish & Wildlife Service 2018). Saltwater areas were identified as all locations that had the saltwater, tidal regime subgroup and included the subtidal, irregularly exposed, regularly flooded, and irregularly flooded water regimes. Permanent freshwater sources were those that were classified as Non-tidal in their regime subgroup and had the permanently flooded, intermittently exposed, or semi permanently flooded water regimes as all of these represented areas where fresh water covered the area for all or most of the year. In addition to these sources of permanent freshwater, the drainage ditches around SH100 were included because they had flowing water throughout most of the year. To identify drainage ditches, features that were clear linear features in the Wetlands Inventory and had the excavated tag were manually selected using ArcMap 10.6. The locations of these drainage ditches were confirmed using published maps available from the Cameron County Drainage District (Cameron County Drainage District #1 2010). A three-m buffer was created around the drainage network to capture the full width of the canals.

To identify agricultural and developed areas, we manually digitized a ESRI orthoimage (year taken: 2018). Older orthoimages taken at different times of year were used to confirm agricultural areas. Developed areas included all buildings, windmills, power stations, and utility towers; roads were digitized separately. Locations of buildings and agricultural areas were confirmed using the Cameron County parcel information (Cameron CAD 2020) and by visits to sites. The majority of roads in the study area were identified from the TxDOT roads database (Texas Department of Transportation 2020), however there were several new dirt roads associated with the San Roman Wind Farm that were digitized manually. A 20 m buffer was created around all paved roads to encompass the full road area as well as the right-of-way while a 10 m buffer was used around all dirt roads.

Using the Reclassify and Raster Calculator tools in ArcGIS 10.6, the classified vegetation map was combined with the water, agriculture, and developed land use layers. Saltwater was given the highest priority and was always on top of other land uses. Buildings, paved roads, and dirt roads were combined into a single class called “developed” and were given the next highest priority, followed by agricultural areas and permanent freshwater. This gave seven classes: saltwater, freshwater, developed, agriculture, forested, shrubs, and open.

The survey transect was divided up into 151 100 m segments and we created a 100-m buffer around each segment. This scale was chosen because WRM risk has been shown to be associated with the presence of specific habitat features such as freshwater sources, access to the road, or movement corridors (Červinka *et al.* 2015; Grilo *et al.* 2016) and this distance was expected to be small enough to assess these effects. In addition, gaps in the fence are highly localized features and would not be expected to influence WRM clustering at large spatial scales. We calculated the proportions of each landcover class within each buffer for each of the segments using an iterative version of the Tabulate Area tool in ArcMap 10.6.

2.4 Statistical Methods

To assess how WRMs changed with construction of mitigation structures, road mortalities were first divided into 13 four-month time blocks. We used an ANOVA in R version 3.6.0 (R Development Core Team 2019) to compare differences in the number of WRMs before, during, and after construction. We categorized the four-month blocks by time period with blocks assigned to the time period in which the majority of dates in the block fell. The number of WRMs in each block were scaled by number of survey days in each time block to account for differences in survey frequency between periods. We applied a log transformation to ensure normality and homoscedasticity. We ran a Shapiro-Wilk test and Levene's test in R to test for normality and homoscedasticity, respectively. We performed post-hoc tests using the Tukey Honestly Significant Difference test in R.

We ran Emerging Hot Spot Analysis (EHSA) based on the tool of the same name available in ArcGIS to assess how the location and strength of WRM clusters changed through time, (Harris *et al.* 2017). The ArcGIS tool was modified so we could use the space blocks used to identify the percentage of land cover. These blocks better fit the highway than the tool-created blocks and could accommodate areas with zero WRMs throughout the entire study period. Wildlife road mortalities divided up by time period; the count of WRMs for each time period in each space-block was calculated using the Spatial Join tool in ArcGIS. We ran the Local Hot Spot Analysis tool in ArcMap 10.6 on each time block using a 500 m fixed distance threshold and the Euclidean distance method for calculating distance between locations (Mitchell 2005). We applied the false discovery rate (FDR) correction to the analyses to account for multiple testing and spatial autocorrelation (Caldas de Castro & Singer 2006). The Local Moran's I tool was also run in ArcMap 10.6 on each time block using the same parameters as the hot spot analysis. Statistical significance was estimated using 9999 permutations. Next, the hot spot results were exported to R and the Mann-Kendall trend test was run on the hot spot z scores using the "mk.test" function in the trend package to assess change in hot spot intensity through time (Pohlert 2018). We performed the FDR correction for the Mann-Kendall test using the "padjust" function in R (R Development Core Team 2019). Spatiotemporal patterns of WRMs were assessed using the defined pattern types for the EHSA and the Local Outlier Analysis tools in ArcGIS (Harris *et al.* 2017).

To assess how distance to fence gaps affected the intensity of WRM clusters, we ran a separate hot spot analysis in ArcMap 10.6 for each of the three construction periods: before, during, and after. The parameters were the same as those for the EHSA. The Z scores, representing cluster intensity, were used as the response variable in linear regressions for each time period.

Distance to fence gaps included distance from the center of the space-block to the nearest wildlife guard, wing wall, and gate, as well as the presence of fencing in most of the space-block. Fence presence was a binary variable and represented whether greater than 50% of a space-block was within the fenced area. Since all the distances and fence presence were highly correlated with each other, the effects of different fence gaps could not be distinguished. Instead, a principal components analysis (PCA) was run to include all four factors. The “prcomp” function in R was used to identify the proportion of variance explained for each principal component axis (R Development Core Team 2019). The first PC axis explained 85% of the variation so it was the only axis included in the regression.

For each time period, hot spot Z score was regressed against fence gap PC axis 1, forested proportion, shrub proportion, open proportion, agriculture proportion, developed proportion, and freshwater proportion. The saltwater class did not appear in any of the buffers, so this class was excluded. No interactions were tested because it was not expected that the proportion of land use would affect how distance to fence gaps influence mortality concentration. While road characteristics such as traffic volume, road size and type, and speed limit may also impact WRMs (Clevenger, Chruszcz & Gunson 2001; Grilo, Ferreira & Revilla 2015), on SH100, there were only minor changes in these characteristics along the highway so none of these characteristics were included.

Using the MuMIn package in R, model selection and model averaging were performed to assess the relationship between WRM concentration and fence gaps and surrounding habitat (Burnham & Anderson 2002; Barton 2013). Models that were within two ΔAICc values of the best model were used for averaging. McFadden pseudo R^2 values for the individual models were calculated using the pscl package in R (Jackman 2012).

3 Results

3.1 Change in Wildlife Road Mortalities through Time

In total, we surveyed 3360 km of road and identified 390 target species WRNs (13-44 per time block) and 376 non-target WRMs (10-60) (Table 2). Most target species WRMs were mammals with Virginia opossums (*Didelphis virginiana*), eastern cottontails (*Sylvilagus floridanus*), and northern raccoons (*Procyon lotor*) making up the majority of WRMs throughout all time periods (Appendix A). There was greater variation in WRMs/survey day in the before construction period when only two surveys were conducted per month than in either of the other periods when more surveys were conducted (Fig. 2). Visually, the majority of WRMs occurred on the western side of the survey transect, an area with most of the wildlife crossings and fence gaps (Fig. 3). Overall, there were significant differences in the log of number of WRMs before, during, and after construction of wildlife mitigation structures ($F_{2,10} = 10.88$, $P = 0.003$; Table 3). Post-hoc tests revealed that there were significantly more WRMs before construction than during construction ($t = -1.314$, $p = 0.002$), however there were not significant differences between the before and after ($t = -0.703$, $p = 0.092$) and the during and after construction periods ($t = 0.611$, $p = 0.125$).

Throughout the entire study period, there were few statistically significant clusters, mostly occurring before and during construction (33 space-time blocks out of a possible 1963 space-time blocks; Fig. 4). There were several non-statistically significant hot spots that occurred throughout the study period, primarily on the western side of the survey transect and in similar locations as most of the WRMs. Additionally, the Mann-Kendall Trend test revealed no statistically significant trends in hot spot z scores through time (Fig. 5). There were several non-significant increasing and decreasing trends distributed throughout the survey transect.

The local Moran's I tests showed that there were several locations where clustering of both high numbers of WRMs and low numbers of WRMs occurred (Fig. 6). There were several locations where spikes in WRMs occurred (High-Low clusters), as well as some places where fewer than expected WRMs occurred (Low-High clusters). Most High-Low outliers occurred on the eastern side of the survey transect while most of the Low-High outliers occurred on the western side of the survey transect. Before construction, there was also an area of High-High clustering which was in the same area as a statistically significant hot spot.

3.2 Impacts of Fence gaps on Mortality Trends

The PCA of distance to fence gaps indicated that approximately 85% of the variation among fence gap types was explained along the first PC axis, 8.0% on the second axis, 4.1% on the third, and 2.5% on the fourth (Fig. 7). Along the first PC axis, distance to wildlife guards, wing walls, and gates increased in the same direction while fence presence increased in the opposite direction.

Seven predictors were included in the final models giving a total of 128 possible models. All seven predictors were included in the averaged model for models in all time periods (Table 4). The number of models included in the averaged model ranged from two (after-construction period) to five (before- and during-construction periods). The range of pseudo R^2 varied from 0.042-0.044 for the before-construction model to 0.149 - 0.159 for the during-construction model.

The first PC axis was a significant positive predictor of before, during, and after construction WRM cluster intensity (Table 5). Therefore, as distance to wildlife guards, wing walls, and gates decreased, the intensity of WRM clusters increased, and intensity was greater within the fenced area. The habitat variables (proportion of open, shrubs, and forested) had a significant negative effect on WRM cluster intensity in all time periods with the exception of open before construction (Table 6).

Proportions of developed and agriculture only had an effect in the after-construction period (Table 5). Wildlife road mortality cluster intensity increased in areas with lower proportions of developed and agriculture (Table 6).

4 Discussion

Overall, we found that neither the number of WRMs nor the intensity of WRM clusters changed with construction of the wildlife mitigation structures on SH100. In the before, during, and after-construction periods, there were stronger clusters near areas that had a fence gap in the after-construction period, indicating no change in the spatial distribution of road mortalities. All our analyses agreed, indicating that, as of 1.5 years after construction of mitigation structures on SH100, WRMs have not yet decreased. There was a non-significant decrease in the number of WRMs and fewer statistically significant clusters in the after-construction period so WRMs appear to be trending down; with more time, we may see significantly fewer WRMs. Previous studies have shown that it may take years for wildlife to regularly use wildlife crossings (Clevenger & Waltho 2005). It is possible that as wildlife become familiar with wildlife crossings, we will see fewer animals attempting to cross on the road surface and fewer WRMs as a result.

Despite finding no significant changes in WRM patterns after construction of exclusionary fencing and wildlife crossings, we still draw several interesting conclusions from the analyses. First, there appeared to be a geographical disparity between both WRM counts and clusters along the length of the transect. Second, more WRMs appeared to be occurring in places where there were more fence gaps. Finally, EHSA can provide a unique picture of how WRM patterns change over time.

4.1 Wildlife Road Mortality Distribution along SH100

Despite not finding statistically significant patterns in WRM concentration, the patterns observed may still be important. Most WRMs throughout all time blocks occurred on the western end of the survey transect, an area mostly consisting of private lands and thornscrub habitat on Laguna Atascosa National Wildlife Refuge with fewer WRMs occurring around parts of the survey transect with more open vegetation. One possible explanation for this is that there were fewer animals living around the eastern end of the survey transect. This area was made up primarily of oxeye daisy prairie, cord grass prairie, and salt marsh (Elliott *et al.* 2014) which tended to have fewer species and fewer individuals than forested habitats in South Texas (Yamashita 2020). The western side of the transect was primarily agricultural and forested habitat and both of these have been shown to be associated with greater WRM rates (Puglisi, Lindzey & Bellis 1974; Smith-Patten & Patten 2008; Ascensão *et al.* 2017). Therefore, it is possible that WRM rates may be similar along the length of the survey transect. Additionally, wildlife living in disturbed habitats (such as those near agricultural lands) may be more willing to use road rights-of-way than individuals living in more natural habitats, increasing their risk of vehicle caused mortality (Forman *et al.* 2003).

In 2018 and 2019, there were favorable environmental conditions for population growth in many wildlife species in the study area which may have contributed to the lack of change seen in total WRM rates. Wildlife mitigation structures like exclusionary fencing and wildlife crossings have been shown to increase wildlife populations living around roads (van der Ree, Smith & Grilo 2015) so the combination of favorable growth conditions and mitigation structures may have led to a decrease in the per capita WRM rate. Caceres (2011) showed that, in Brazil, abundance was the most significant predictor of WRM counts so natural increases in animal abundance around SH100 may have led to increases in WRMs after construction. Therefore, the lack of change seen in WRM rates may reflect increased wildlife populations rather than an ineffectiveness of mitigation structures. If wildlife populations are increasing around SH100 and wildlife crossings become more effective with time, then we would expect the decreasing trend in WRMs to continue.

Another contributing factor may be that there were more fence gaps on the western side of the survey transect than the eastern side. While this does not explain the high numbers of WRMs before or during construction, it may have contributed to the lack of decrease in WRMs after construction. The western side of the transect had 12 of 18 wildlife guards, 10 of 16 gates, and two of three wing walls offering multiple places for wildlife to access the road. The effects of different types of fence gaps were not examined in the present study, so it is possible that WRM concentrations may be higher around more permeable gaps such as wing walls or WGs. Therefore, these mitigated fence gaps may not be effective at reducing wildlife access to the road.

4.2 Fence Gaps and Road Mortality

While intensity of WRM clusters was greater near fence gaps, this was true in the before- and during-construction periods when there were no fences or fence gaps. Cserkész *et al.* (2013) examined how WRM counts on a fenced highway was affected by distance to highway interchanges and demonstrated fencing funneled animals towards fence gaps. However, we found no evidence fencing funneled animals onto SH100. Fence gaps along SH100 occurred at high frequency (n = 37 over 12 km of highway) compared to the Cserkész *et al.* (2013) (n = 79 over 640 km) creating more access points and diffusing crossings across several kilometers of road instead of a single access point.

Our study indicated that the spatial pattern of WRMs has not changed with construction of fencing and that WRM clustering increased near fence gaps in all time periods indicating fence gaps may be located in places previously used as wildlife travel corridors. In the after-construction period, fence gaps probably represented known access points and likely had the highest chance of an animal crossing, similar to what McCollister and van Manen (2010) found after construction of wildlife mitigation structures in North Carolina. Fence gaps represent a narrow access point so assessing how they impact WRMs requires a local scale analysis (Červinka *et al.* 2015). At broader scales, the influence of access points to the highway may become masked by landscape level effects such as land cover and the presence of fresh water (Yamashita 2020).

Finally, this study was conducted less than two years after the completion of mitigation structures and it has been shown that wildlife may take several years to adjust to the presence of wildlife crossings (Clevenger 2005; Clevenger & Waltho 2005). It is possible that animals along SH100 were still in the "learning" phase and WRMs, especially around wildlife crossings, may begin to decrease as time passes. There is some visual evidence of this already with only three mortalities occurring within 200 m of crossings 1, 3, 3A, and 4 in the final two-time blocks (eight months; Fig. 3). However, it is unclear if this was a result of learning or chance. Around wildlife crossing 2, the large number of fence gaps near the crossing may increase the amount of time it takes wildlife to learn to use the crossing.

4.5 Using Road Mortality Clusters to Examine Road Mortality Patterns

Using EHSA to examine patterns of WRMs allows one to determine the statistical significance of visually identified WRM hot spots. Knowing whether or not a cluster is significant can have important management implications because wildlife crossings can be expensive when they are built as a stand-alone project (Huijser *et al.* 2009). Solely using number of WRMs may miss important clustering of fewer WRMs which may benefit more from a wildlife crossing (Teixeira *et al.* 2017). Emerging hot spot analysis provides a framework for examining fine scale spatial and temporal patterns of WRMs enabling assessment of how fine scale changes (i.e. wildlife mitigation structures) along a highway affect WRM patterns. This type of analysis can help determine how effective different mitigation structures are, an important question for managers and transportation agencies. Complementing this analysis with monitoring of wildlife mitigation structures using camera traps or another monitoring technique can allow managers to obtain a complete assessment of how wildlife mitigation structures benefit the animal community. Finally, EHSA can provide useful visualizations of WRM data that can help display patterns hidden at larger scales. Generally, WRMs need to be examined at broad spatial and temporal scales due to sample size limitations. These analyses can miss important patterns occurring at finer scales (Levin 1992). While EHSA likely has low power to detect changes in clustering due to low sample sizes in WRM datasets, it can provide useful representations of data that may elucidate previously unknown patterns in WRM datasets. For example, it would have been impossible to see that WRMs appeared to be declining around wildlife crossings 1, 3, 3a, and 4 without the visualizations produced by EHSA.

While EHSA provides several benefits, performing the analysis effectively requires balancing sample size limitations of the WRM dataset with the minimum spatial and temporal resolution required for the hot spot analysis and Mann-Kendall test. Generally, assessments of local hot spots or local clustering require large sample sizes to detect significant clusters (Caldas de Castro & Singer 2006; Grubesic, Wei & Murray 2014). For analysis purposes, medium to large mammal WRM rates tend to be fairly low (Ascensão *et al.* 2017). Therefore, unless one is working in an area with many WRMs or they have a long-term dataset, the power of hot spot analysis may be too low to detect significant WRM hot spots in medium to large mammals. This is especially true for local hot spot analysis when one must apply a correction for multiple testing and spatial autocorrelation (Caldas de Castro & Singer 2006).

The Mann-Kendall test requires a minimum of 10 time blocks to run (Hipel & Mcleod 2005; Harris *et al.* 2017). In order to meet this requirement, we divided WRMs into four-month time periods. This meant that the total number of WRMs used to identify clustering for each time block (range 21-44) was likely too low to detect significant changes in clustering through time (Caldas de Castro & Singer 2006; Grubesic, Wei & Murray 2014). Therefore, an assessment of how sample size affects the power of local hot spot analysis will be required before this method can be applied more broadly.

4.6 Conclusions

We present a novel method for assessing how the construction of wildlife mitigation structures modifies the distribution of WRMs. Unfortunately, the ability of EHSA to explain variation in WRMs may be limited by the number of WRMs, which in this study, was low through all time periods. Emerging hot spot analysis in WRM studies can provide a useful snapshot of how patterns change through time, but appears to perform powerful statistical tests unless larger WRM datasets are available which can only be obtained through long-term datasets or very long survey transects. We recommend transportation managers conduct long-term WRM surveys, especially in areas where mitigation structures such as wildlife crossings are employed to document whether WRMs are reduced.

By combining EHSA results with comparisons of the before-, during-, and after-construction periods, we were able to demonstrate that the construction of exclusionary fencing and wildlife crossings did not change patterns of WRMs, possibly because fence gaps were located in places where WRM concentration was high before construction. Visual inspection of fine scale WRM patterns, available from EHSA, revealed that WRMs may be decreasing around wildlife crossings on SH100, indicating that animals may be learning that wildlife crossings provide a safer passageway across roads than the road surface. Therefore, EHSA can provide useful insights into how changes in the roadway impact wildlife use of the road area.

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Author Contributions

TY, KR, JY, and RK conceived the ideas and designed the methodology; TL, KR, and TY collected the data; TY analyzed the data; TY and RK led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Original road mortality data: Open Science Framework: https://osf.io/eqpvj/

References

Allen, T.D.H., Huijser, M.P. & Willey, D.W. (2013) Effectiveness of wildlife guards at access roads. *Wildlife Society Bulletin,* **37,** 402-408.

Andis, A.Z., Huijser, M.P. & Broberg, L. (2017) Performance of Arch-Style Road Crossing Structures from Relative Movement Rates of Large Mammals. *Frontiers in Ecology and the Environment,* **5**.

Anselin, L. (1995) Local Indicators of Spatial Association—LISA. *Geographical Analysis,* **27,** 93-115.

Ascensão, F., Desbiez, A.L.J., Medici, E.P. & Bager, A. (2017) Spatial patterns of road mortality of medium–large mammals in Mato Grosso do Sul, Brazil. *Wildlife Research,* **44,** 135-146.

Barton, K. (2013) MuMIm: Multi-model inference. R package version 1.9.13.

Bíl, M., Andrášik, R., Duľa, M. & Sedoník, J. (2019) On reliable identification of factors influencing wildlife-vehicle collisions along roads. *Journal of Environmental Management,* **237,** 297-304.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference : A Practical Information-theoretic Approach*. Springer, New York.

Caceres, N.C. (2011) Biological characteristics influence mammal road kill in an Atlantic Forest–Cerrado interface in south-western Brazil. *Italian Journal of Zoology,* **78,** 379-389.

Caldas de Castro, M. & Singer, B.H. (2006) Controlling the False Discovery Rate: A New Application to Account for Multiple and Dependent Tests in Local Statistics of Spatial Association. *Geographical Analysis,* **38,** 180-208.

Cameron CAD (2020) *Cameron County Parcels*. Cameron County Appraisal District, San Benito, TX, USA.

Cameron County Drainage District #1 (2010) *Cameron County Drainage District NO. 1 District map*. Cameron County Drainage District NO. 1, Brownsville, TX, USA.

Červinka, J., Riegert, J., Grill, S. & Šálek, M. (2015) Large-scale evaluation of carnivore road mortality: the effect of landscape and local scale characteristics. *Mammal Research,* **60,** 233-243.

Clevenger, A., Chruszcz, B. & Gunson, K. (2001) Highway mitigation fencing reduces wildlife-vehicle collisions. *Wildlife Society Bulletin,* **29,** 646-653.

Clevenger, A.P. (2005) Conservation value of wildlife crossings: Measures of performance and research directions. *Gaia-Ecological Perspectives for Science and Society,* **14,** 124-129.

Clevenger, A.P., Chruszczc, B. & Gunson, K.E. (2003) Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. *Biological Conservation,* **109,** 15-26.

Clevenger, A.P. & Waltho, N. (2005) Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. *Biological Conservation,* **121,** 453-464.

Cserkész, T., Ottlecz, B., Cserkész-Nagy, Á. & Farkas, J. (2013) Interchange as the main factor determining wildlife–vehicle collision hotspots on the fenced highways: spatial analysis and applications. *European Journal of Wildlife Research,* **59,** 587-597.

Elliott, L.F., Diamond, D.D., True, D., Blodgett, C.F., Pursell, D., German, D. & Treuer-Kuehn, A. (2014) Ecological Mapping Systems of Texas: Summary Report. Texas Parks & Wildlife Department, Austin, Texas.

Environmental Affairs Division (2015) Biological Assessment (BA) for Consultation with the USFWS: SH 100 from 4.0 miles east of FM 1847 to 1.75 miles west of FM 510, Cameron County. Texas Department of Transportation.

ESRI (2017) ArcGIS Desktop: Release 10.6. Environmental Systems Research Institute, Redlands, CA.

Forman, R., T. T., Sperling, D., Bissonette, J.A., Clevenger, A.P., Cutshall, C.D., Dale, V.H., Fahrig, L., France, R., Goldman, C.R., Heanue, K., Jones, J.A., Swanson, F.J., Turrentine, T. & Winter, T.C. (2003) *Road Ecology: science and solutions*. Island Press, Washington D.C.

Getis, A. & Ord, J.K. (1992) The Analysis of Spatial Association by Use of Distance Statistics. *Geographical Analysis,* **24,** 189-206.

Grilo, C., de Resende Cardoso, T., Solar, R. & Bager, A. (2016) Do the size and shape of spatial units jeopardize the road mortality-risk factors estimates? *Natureza & Conservação,* **14,** 8-13.

Grilo, C., Ferreira, F.Z. & Revilla, E. (2015) No evidence of a threshold in traffic volume affecting road-kill mortality at a large spatio-temporal scale. *Environmental Impact Assessment Review,* **55,** 54-58.

Grubesic, T.H., Wei, R. & Murray, A.T. (2014) Spatial Clustering Overview and Comparison: Accuracy, Sensitivity, and Computational Expense. *Annals of the Association of American Geographers,* **104,** 1134-1156.

Harris, N.L., Goldman, E., Gabris, C., Nordling, J., Minnemeyer, S., Ansari, S., Lippmann, M., Bennett, L., Raad, M., Hansen, M. & Potapov, P. (2017) Using spatial statistics to identify emerging hot spots of forest loss. *Environmental Research Letters,* **12,** 024012.

Hipel, K.W. & Mcleod, A.I. (2005) *Time Series Modelling of Water Resources and Environmental Systems*. Elsevier Science B.V., Amsterdam, Netherlands.

Huijser, M.P., Duffield, J.W., Clevenger, A.P., Ament, R.J. & McGowen, P.T. (2009) Cost-Benefit Analyses of Mitigation Measures Aimed at Reducing Collisions with Large Ungulates in the United States and Canada: a Decision Support Tool. *Ecology and Society,* **14**.

Huijser, M.P., Fairbank, E.R., Camel-Means, W., Graham, J., Watson, V., Basting, P. & Becker, D. (2016) Effectiveness of short sections of wildlife fencing and crossing structures along highways in reducing wildlife–vehicle collisions and providing safe crossing opportunities for large mammals. *Biological Conservation,* **197,** 61-68.

Jackman, S. (2012) Pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University.

Levin, S.A. (1992) The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. *Ecology,* **73,** 1943-1967.

Livingston, T. (2019) Wildlife Road Mortality Survey Methodologies. Master of Science Masters, University of Texas Rio Grande Valley.

McCollister, M.F. & van Manen, F.T. (2010) Effectiveness of Wildlife Underpasses and Fencing to Reduce Wildlife–Vehicle Collisions. *Journal of Wildlife Management,* **74,** 1722-1731.

Mitchell, A. (2005) *The ESRI Guide to GIS Analysis Volume 2: Spatial Measurements & Statistics*. ESRI Press, Redlands, CA, USA.

Ord, J.K. & Getis, A. (1995) Local Spatial Autocorrelation Statistics: Distributional Issues and an Application. *Geographical Analysis,* **27,** 286-306.

Pohlert, T. (2018) *Trend: Non-Parametric Trend Tests and Change-Point Detection. R package version 1.1.1*.

Puglisi, M.J., Lindzey, J.S. & Bellis, E.D. (1974) Factors Associated with Highway Mortality of White-Tailed Deer. *The Journal of Wildlife Management,* **38,** 799-807.

R Development Core Team (2019) *R: A Language and Environment for Statistical Computing. Version 3.6.0*. R Foundation for Statistical Computing, Vienna, Austria.

Smith-Patten, B.D. & Patten, M.A. (2008) Diversity, Seasonality, and Context of Mammalian Roadkills in the Southern Great Plains. *Environmental Management,* **41,** 844-852.

Snow, N.P., Williams, D.M. & Porter, W.F. (2014) A landscape-based approach for delineating hotspots of wildlife-vehicle collisions. *Landscape Ecology,* **29,** 817-829.

Teixeira, F.Z., Kindel, A., Hartz, S.M., Mitchell, S. & Fahrig, L. (2017) When road-kill hotspots do not indicate the best sites for road-kill mitigation. *Journal of Applied Ecology,* **54,** 1544-1551.

Texas Department of Transportation (2019) *Texas Department of Transportation Annual Average Daily Traffic Annuals*. Texas Department of Transportation, Austin, TX.

Texas Department of Transportation (2020) *TxDOT Roadway Inventory*. Transportation Planning and Programming Division, Texas Department of Transportation, Austin, Texas, USA.

U.S. Fish & Wildlife Service (2018) *National Wetlands Inventory Program Overview*. United States Fish and Wildlife Service, Washington DC, USA.

van der Ree, R., Smith, D.J. & Grilo, C. (2015) *Handbook of Road Ecology*. John Wiley & Sons, Ltd., Chichester, Sussex, UK.

Yamashita, T. (2020) The Influence of Human Disturbance on Wildlife Use of a Highway in South Texas. Master of Science, University of Texas Rio Grande Valley.

Figures and Tables

Table 1: Comparison of wildlife road mortality survey methodologies among construction periods on State Highway 100, Cameron County, Texas. Surveys per month are the approximate number of wildlife road mortality surveys conducted per month, time period is the dates that surveys were being conducted, and carcass removal indicates if carcasses were marked or moved by surveyors.

	Before	**During**	**After**
Surveys/month	2	8	4
Time period	Aug 2015 – Aug 2016	Sep 2016 – May 2018	Jun 2018 – Sep 2019
Vehicle Speed	40 kmh	48 – 64 kmh	48 – 64 kmh
People/Vehicle	2	2	2
Coordinates	GPS	GPS	GPS
Photograph	No	Yes	Yes
Carcass removal	Marked but not removed	Unmarked and not removed	Unmarked and not removed
Taxa recorded	Mammalia, Reptilia	All	All

Table 2: Total number of wildlife road mortalities by class before, during, and after construction of wildlife mitigation structures on State Highway 100, Cameron County, Texas. For a complete breakdown of wildlife road mortalities by species and time period, see Appendix A.

Group	Class	Before	During	After	Total Mortalities
	Months of Data	11	20	16	------------
Target Species	Mammalia	89	140	114	343
	Reptilia	28	4	16	48
	Total	117	144	130	391
Non-target Species	Aves	5*	50	101	156
	Mammalia	36	12	25	73
	Reptilia	67	19	40	126
	Malacostraca	0	0	6	6
	Unknown	1	0	1	2
	Total	109	81	186	376
	Grand Total	226	225	316	767

Table 3: ANOVA table comparing the log of number of wildlife road mortalities on State Highway 100, Cameron County, Texas in the before, during, and after construction periods. Post-hoc tests were performed using the Tukey's Honestly Significant Difference test. The test statistic for the ANOVA was an F; post-hoc testsused T values.

Variable	Sum Squares	Mean Squares	df	Statistic	P value
Time Period	3.837	1.918	2	10.88	0.003
Before – During				1.31	0.002
Before – Post				0.70	0.092
During – Post				-0.61	0.125
Residuals	1.762	0.176	10		

Table 4: Summary of the averaged regression models for the effect of land cover and fence gaps on the intensity of wildlife road mortality clustering along State Highway 100, Cameron County, Texas. The factors included in the model were the distance to fence gaps Principal Components axis (Gap), open vegetation (Open), shrubs (Shrub), forested, developed, agriculture, and fresh water (Water). The "models included" are the number of models used to compute the model averaged estimates and p values. Significance of a factor is indicated by a "+" (positive effect) or a "-" (negative effect). The pseudo R2 range is the range of McFadden pseudo R2 values for each model included in the averaged model.

	Time Period		
	Before	During	After
Models Included	5	5	2
Fence Gap	+	+	+
Open	NS	-	-
Shrub	-	-	-
Forested	-	-	-
Developed	NS	NS	-
Agriculture	NS	NS	-
Water	NS	NS	NS
Pseudo R^2 Range	0.042 - 0.044	0.149 - 0.159	0.122 - 0.124

Table 5: Full model summaries for the averaged regression model assessing the effects on wildlife road mortality clustering on State Highway 100, Cameron County, Texas for before, during, and after construction periods showing the estimated effect, standard error, Z score, and P value. Significant effects are bolded.

Time Period	Variable	Estimate	Adjusted SE	Z score	P value
Before	(Intercept)	0.538	0.267	2.019	**0.044**
	PC1	0.129	0.056	2.304	**0.021**
	Forested	-3.138	1.489	2.108	**0.035**
	Shrubs	-2.963	1.022	2.900	**0.004**
	Freshwater	-2.758	8.496	0.325	0.745
	Developed	-0.238	0.738	0.323	0.747
	Agriculture	0.218	0.804	0.271	0.786
	Open	-0.086	0.322	0.266	0.790
During	(Intercept)	1.626	0.482	3.373	**0.001**
	PC1	0.137	0.055	2.504	**0.012**
	Agriculture	-1.384	1.616	0.856	0.392
	Developed	-1.493	1.386	1.077	0.281
	Freshwater	22.296	15.565	1.432	0.152
	Forested	-10.129	1.434	7.064	**0.000**
	Open	-1.445	0.601	2.404	**0.016**
	Shrubs	-2.733	0.972	2.812	**0.005**
After	(Intercept)	2.474	0.393	6.292	**0.000**
	PC1	0.123	0.051	2.396	**0.017**
	Agriculture	-4.281	1.387	3.086	**0.002**
	Developed	-5.243	1.104	4.751	**0.000**
	Forested	-3.677	1.309	2.808	**0.005**
	Open	-1.592	0.563	2.829	**0.005**
	Shrubs	-2.688	0.912	2.947	**0.003**
	Freshwater	3.490	8.814	0.396	0.692

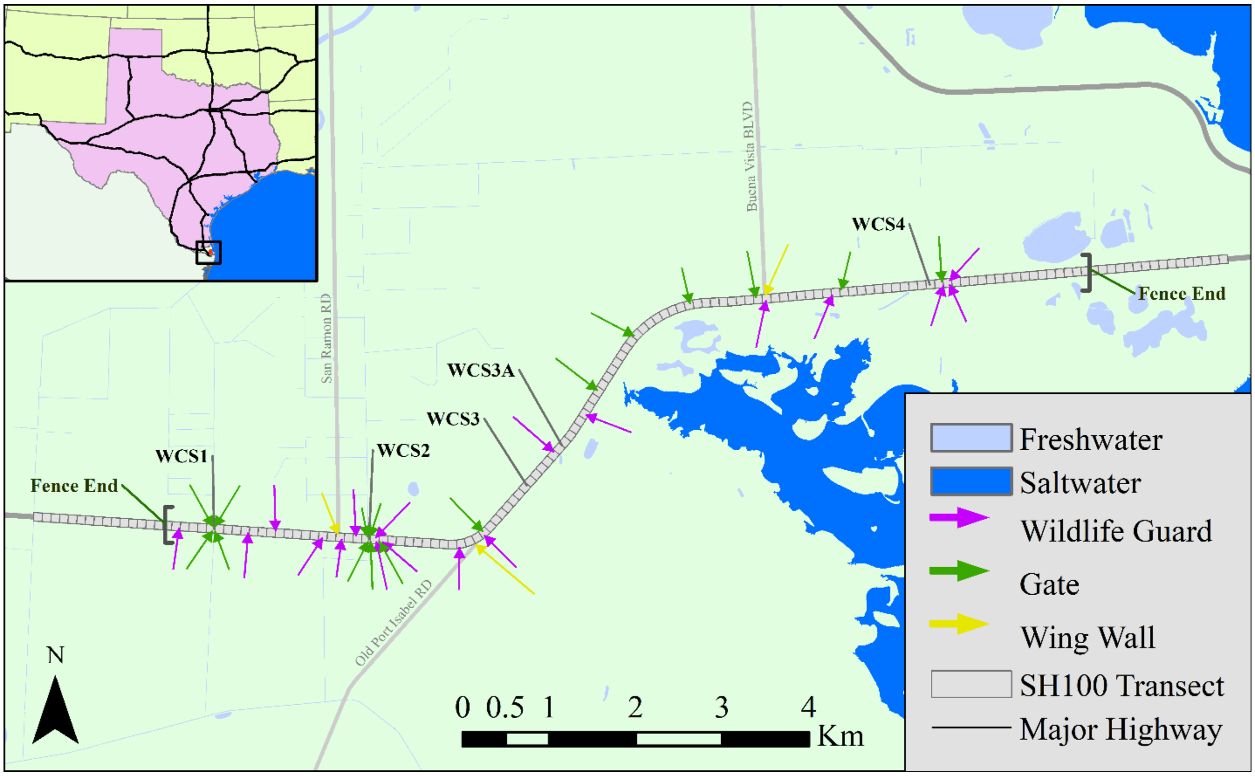

Fig. 1: Map of the wildlife mitigation area on State Highway 100 showing the three types of fence gaps: gates, wildlife guards, and wing walls. The wildlife road mortality survey transect is divided into 151 100 m road segments.

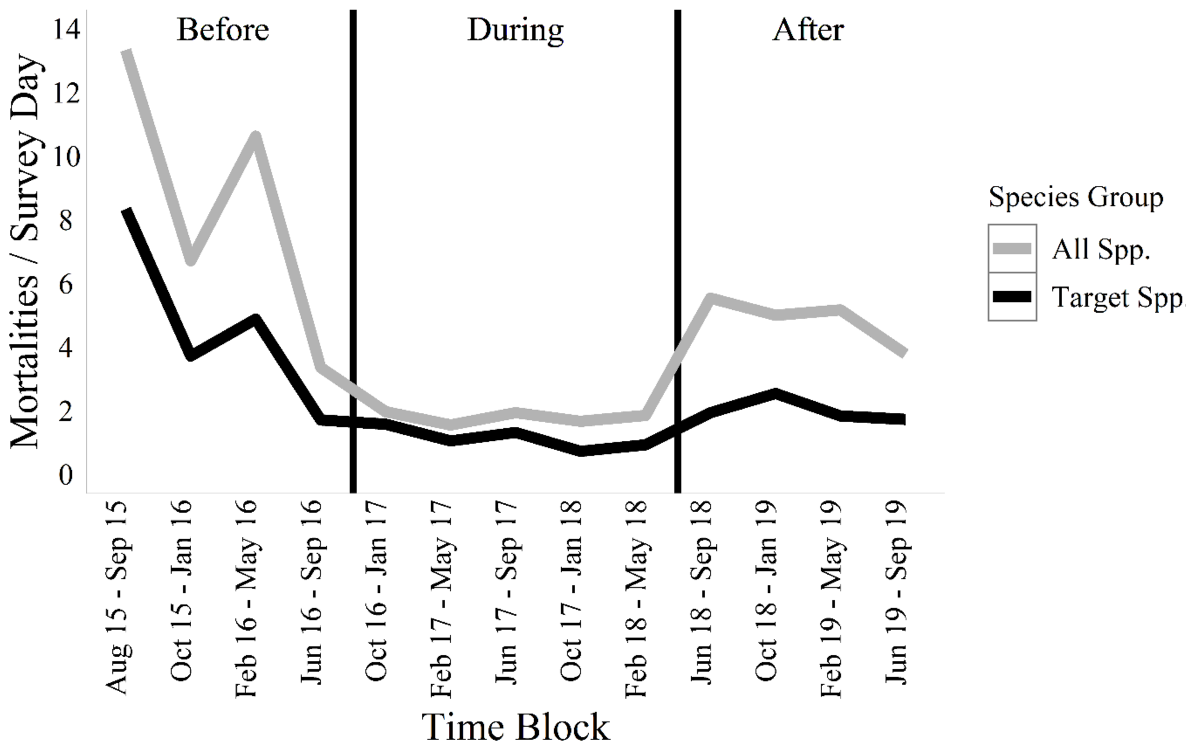

Fig. 2: Total number of wildlife road mortalities per time block normalized by number of survey days along State Highway 100, Cameron County, Texas. Wildlife road mortalities shown include target species (mammals larger than rodents, turtles, and tortoises) and all species combined (target plus non-target species). Vertical lines delineate the periods before, during, and after the construction of wildlife mitigation structures.

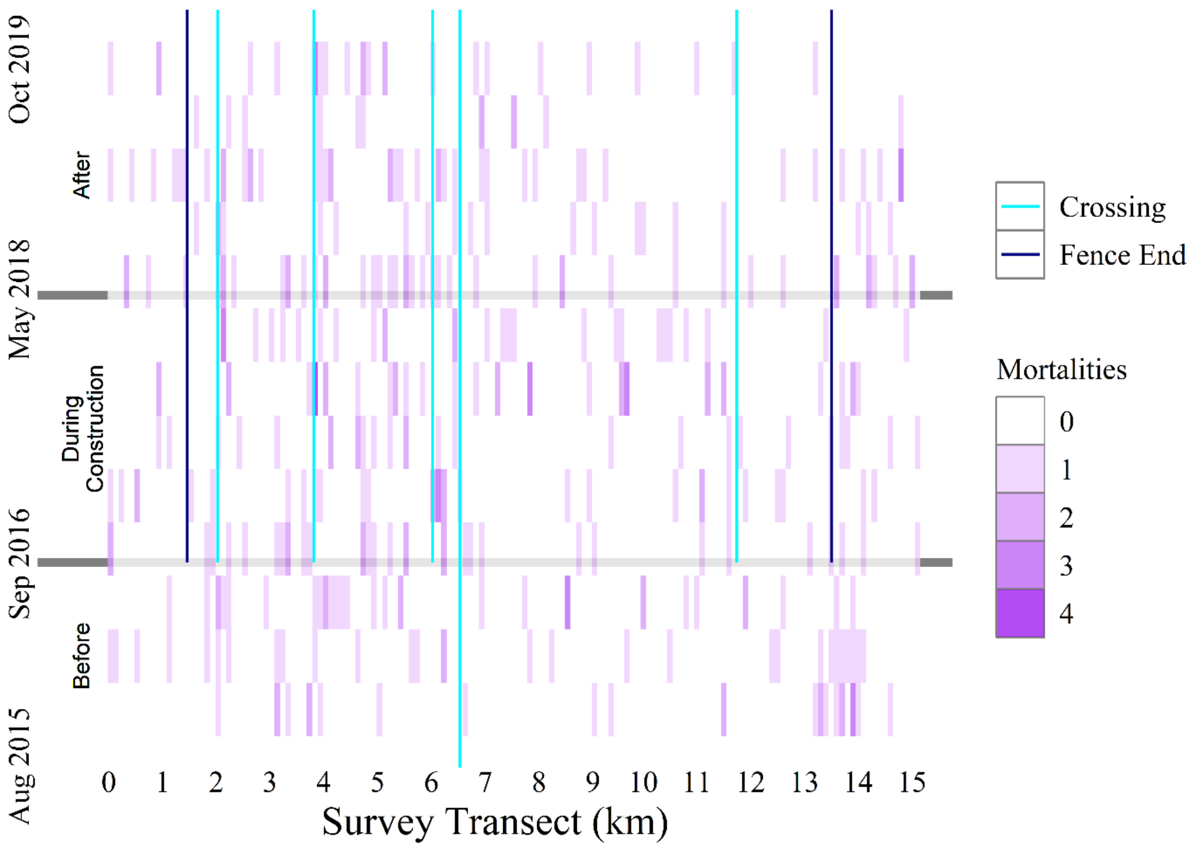

Fig. 3: Number of wildlife road mortalities by space-time block along State Highway 100 (SH100), Cameron County, Texas. SH100 was divided into 151 100 m road segments and 13 time blocks and each block was filled with the number of wildlife road mortalities during that period. The survey transect blocks represent road segments and increase from west to east. To better relate this to the study area map, the approximate locations of wildlife crossings and fence ends are also indicated by vertical lines and the construction periods are indicated by horizontal lines.

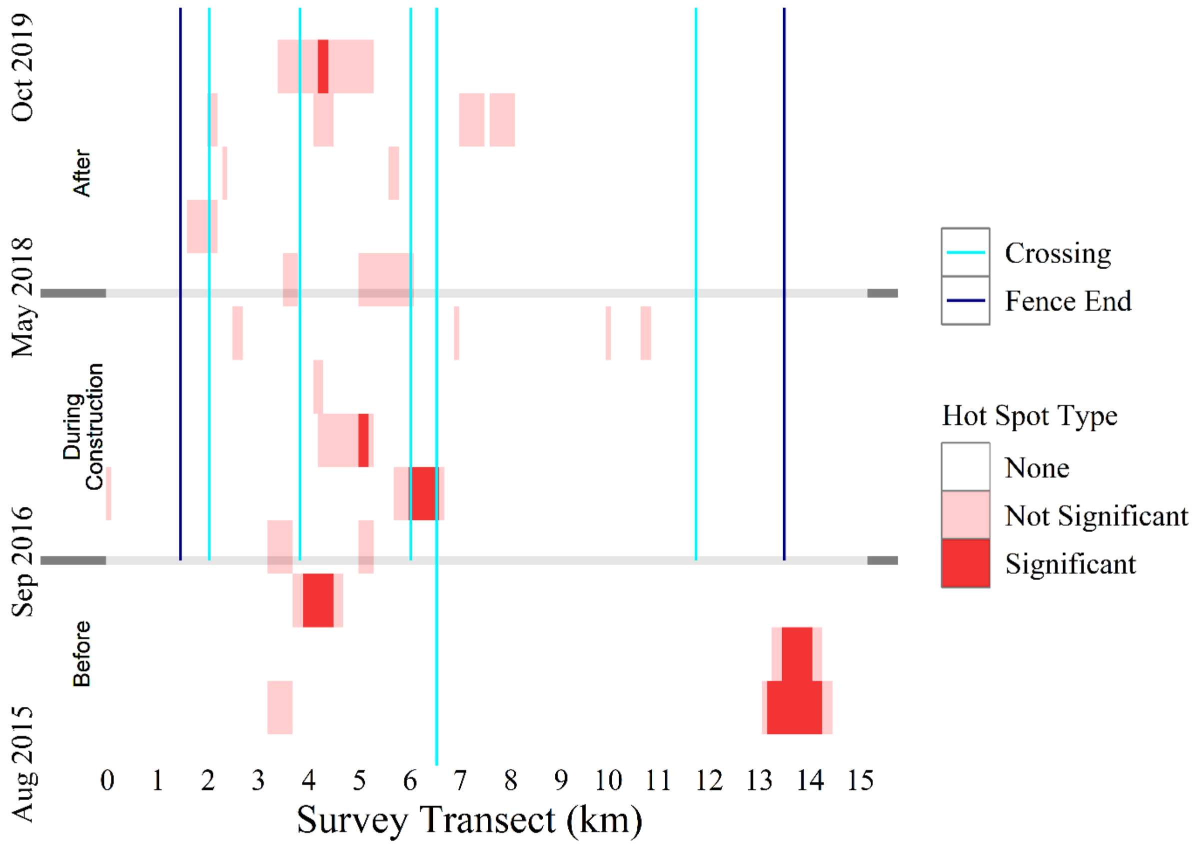

Fig. 4: Heatmap of wildlife road mortality hot spots along State Highway 100, Cameron County, Texas. Statistically significant hot spots are those that were significant after applying the false discovery rate correction, while non-significant hot spots were those that were only significant without the correction. The survey transect blocks represent road segments and increase from west to east. To better relate this to the study area map, the approximate locations of wildlife crossings and fence ends are also indicated by vertical lines and the construction periods are indicated by horizontal lines.

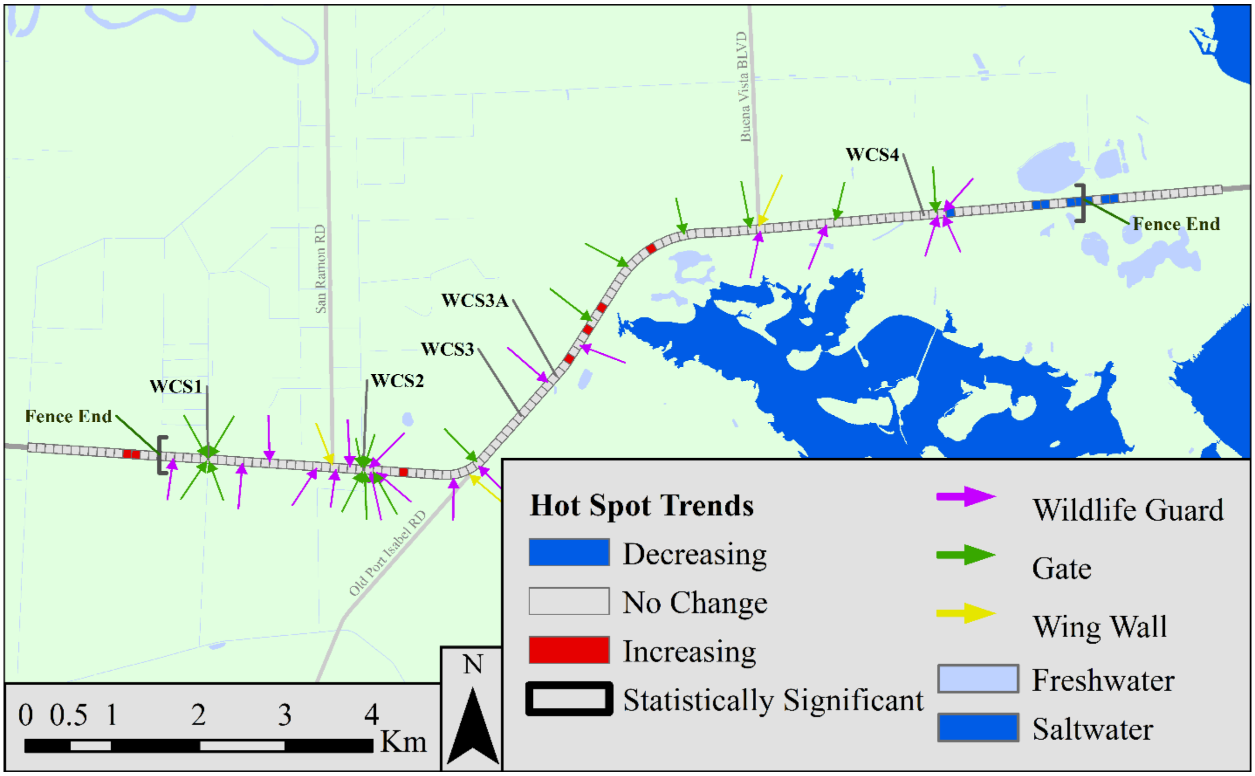

Fig. 5: Trends in the concentration of wildlife road mortalities along State Highway 100, Cameron County, Texas from the Mann-Kendall trend test. Decreasing trends indicate that the concentration of wildlife road mortalities decreased over time while increasing trends indicate that concentration of wildlife road mortalities increased over time. No trends were statistically significant (at $\alpha = 0.05$) after the FDR correction was applied.

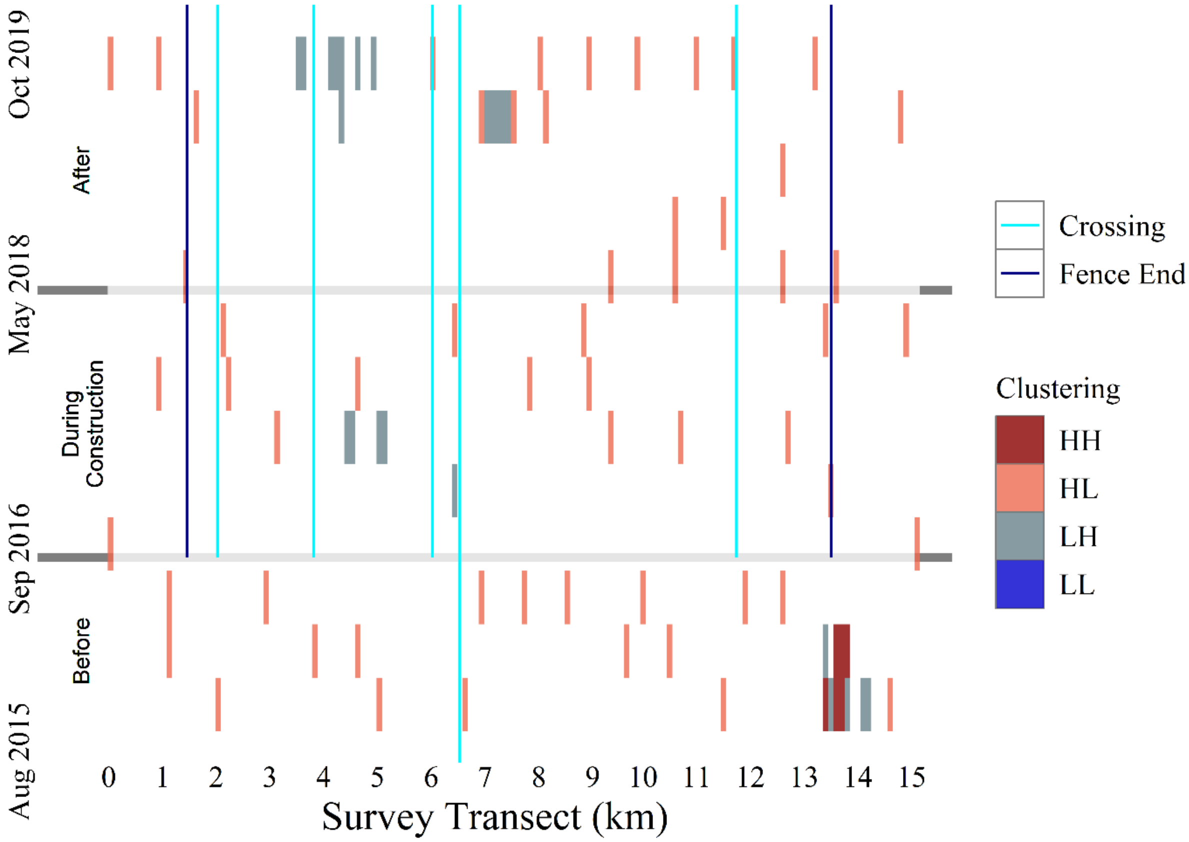

Fig. 6: Heatmap of the local Moran's I clusters of wildlife road mortalities through time along State Highway 100, Cameron County, Texas. HH represents high-high clustering, HL is a high-low outlier, LH is a low-high outlier, and LL represents low-low clustering. The survey transect blocks represent road segments and increase from west to east. To better relate this to the study area map, the approximate locations of wildlife crossings and fence ends are also indicated by vertical lines and the construction periods are indicated by horizontal lines.

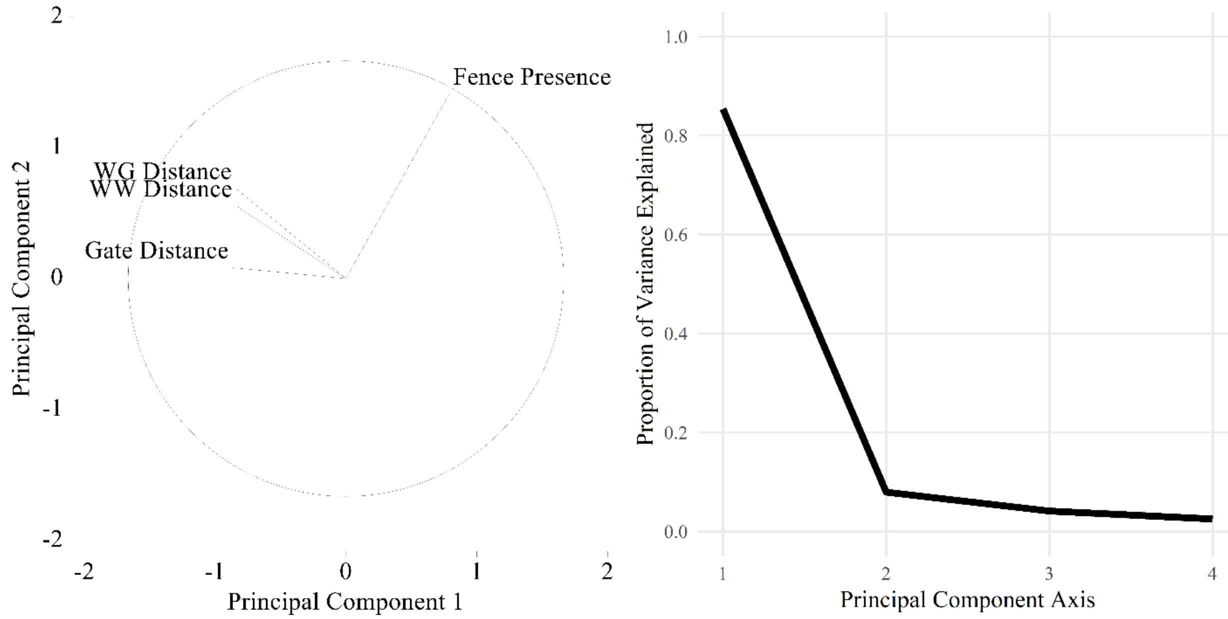

Fig. 7: Principal components plot showing the relationship between distance from a road segment on State Highway 100, Cameron County, Texas to different fence gaps (wildlife guards (WG), wing walls (WW), and gates) and the presence of fencing along the first two principal components axes (left) and a scree plot showing the proportion of variance explained along each Principal Components axis (right).