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Spatiotemporal Patterns and Road Mortality Hotspots of Herpetofauna on a Mediterranean Island

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Abstract: Traffic intensification, often in conjunction with habitat fragmentation, has caused frequent roadkill incidents, particularly among reptiles, amphibians, and other taxa. Herpetofauna species, which are slow moving and habitat dependent, are particularly susceptible to these incidents because they often use roads during thermoregulation. Lesvos, the eighth-largest Mediterranean island, boasts a biodiversity that surpasses most other Mediterranean islands of similar or larger size, with a plethora of herpetofauna species inhabiting its terrain. In recent years, new roads were constructed on Lesvos, which are considered to be one of the most important factors that negatively affect the island's wildlife as they increase the non-natural mortality of animals, are obstacles to their mobility, and reduce the connectivity of populations by limiting their dynamics. In the present study, we examined the road mortality of amphibians and reptiles by analyzing (a) the temporal and seasonal patterns of such incidents, (b) the relationship between roadside habitats and road network characteristics with the roadkilled herpetofauna species, and (c) their spatial distribution on Lesvos during the years 2009–2012 and 2016–2019. To identify significant clusters of reptile and amphibian roadkills, we performed hotspot analysis by utilizing kernel density estimation and Getis Ord Gi* statistics. We recorded a total of 330 roadkills among 20 species, with the highest rates emerging among the European green toad (*Bufotes viridis*), the European glass lizard (*Pseudopus apodus*), the Rhodos green lizard (*Lacerta diplochondrodes*), and the snake-eyed lizard (*Ophisops elegans*). Spatial statistical analysis revealed that roads close to herpetofauna habitats exhibit statistically significant clusters of roadkills that intensify during the spring season. Regular monitoring and mapping of herpetofauna road mortality will enable the implementation of management strategies to mitigate the negative impact of this phenomenon.

Keywords: road ecology; roadkills; reptiles; amphibians; spatial statistics

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1. Introduction

The exponential growth of transportation and development has catalyzed a concomitant proliferation of roadways, which are expected to continue expanding rapidly over the next century [1], on a global scale. This expansion of roads has brought about widespread and multifarious consequences for both the abiotic and the biotic components of ecosystems [2]. In particular, roads can affect the abiotic aspects of landscapes including the hydrology and water quality [3], changes to erosion and deposition rates of sediments [4], the introduction of chemical spills and toxic contaminants [5], and noise pollution [6], as well as other atmospheric effects [7]. Simultaneously, roads are drivers of transformation that induce both primary (or immediate) impacts, as well as secondary (or derivative) impacts on the biotic community. In fact, they have been deemed significant sources of alteration, exerting both direct and indirect effects on the biota [8]. The proliferation of major roadways has led to the fragmentation of the Earth's terrestrial surface into more than 600,000 fragments, with a majority of these being less than 1 km^2 in area and only 7% surpassing 100 km^2 in size [9]. Consequently, the occurrence of wildlife–vehicle collisions has become alarmingly high, with roads being a leading cause of anthropogenic mortality for numerous invertebrates and vertebrates, second only to legal harvesting practices [10]. The direct effects of roads manifest in the form of destructive alterations to animal and plant populations, arising from the complete obliteration of ecosystems along their routes.

The decimation of biodiversity globally is brought about by the phenomenon of road mortality, which has been extensively documented through numerous studies [11–16]. Representatives of all terrestrial [17–20] and flying fauna [21–25] are vulnerable to vehicular impacts, regardless of the road type or region, including residential areas [26,27] and even protected zones [28–32]. The frequency and extent of roadkills are dependent upon various factors, such as seasonal variations [33–35], climatic conditions [36], time of day [37], traffic density [37,38], speed limit [39], the health state [40] and behavior of the animals [40–42], the type of road [38,43], the positioning of the road network within an ecosystem [34,35,44,45], the characteristics of the surrounding habitat [33,38,46], the presence of vegetation on both sides of the roads [23,37], and human activity in the vicinity [47] or exceptional circumstances [48]. While the vast majority of roadkills are accidental, a substantial proportion of deliberate roadkills have also been reported [49,50].

The occurrence of road-related fatalities among wildlife, much like the barrier effect, leads to the division of populations into smaller sub-populations, thereby curtailing inter-subpopulation gene flow and exacerbating the dearth of genetic diversity and reduced reproductive success [51,52]. Species that exhibit lower mobility and a higher propensity for crossing roads are especially prone to this conflict, with heightened mortality rates resulting from vehicular collisions [53,54]. Among all vertebrates, the herpetofauna group is widely considered to be the most vulnerable, largely owing to the unique ecological, physiological, and behavioral attributes of these species [55]. Herpetofauna frequently utilize roads for migration to different habitats in pursuit of breeding sites, mating, and food, and they use roads to a great extent during the process of thermoregulation [41,56–58]. Reptiles and amphibians, renowned for their relatively sluggish movement, are particularly susceptible to vehicular collisions [51]. Furthermore, seasonal movements, such as during the breeding season or in response to changes in temperature and meteorological conditions, are also known to have a detrimental effect on road mortality rates [59,60]. The underlying causes of vehicular mortality among vertebrates are a result of both road-related factors and animal behavior. With regard to the former, research has shown that vehicle speed and traffic volume play crucial roles regarding animal fatalities [61,62]. At the same time, drivers' attitudes and intentions toward different taxonomic groups of animals can also vary [50,63].

The phenomenon of wildlife road mortality has garnered substantial attention in mainland regions worldwide, as evidenced by numerous studies [64–68]. In contrast, despite the crucial ecological significance and sensitivity of islands, there has been a lack of research examining wildlife roadkill incidents in such environments [69–71], particularly with regard to herpetofauna. Greece, a biodiversity hotspot [72,73] and one of the least fragmented countries among the European ones [74], possesses over 6000 islands and islets that harbor abundant and diverse populations of herpetofauna species. Representing 38% of all reptiles in Europe, Greece is home to a substantial number of reptiles, a significant proportion of which are considered threatened at the European level (13%). Greece is also a significant repository of amphibian diversity, accounting for 27% of all amphibians in Europe, with numerous endemic species on the Mediterranean islands [75]. A considerable number of amphibian species in Greece (19%) are considered threatened at the European level, primarily due to the loss and degradation of breeding habitats from agricultural activities [76].

Despite this richness, there is a glaring deficiency in the surveillance and collection of data regarding wildlife road mortality. As far as it can be ascertained, there is a lack of wildlife roadkill studies both on the mainland and on islands; only one study has been published on this subject and pertains exclusively to the brown bear within a designated geographical region from mainland Greece [77].

In light of this information gap, and keeping in mind the increased construction of roads and other linear infrastructure globally, it is essential to understand the impact on wildlife populations and take steps to mitigate their negative effects. In this regard, we present the first specialized examination of the road mortality of herpetofauna species on the Greek island of Lesvos, the eighth-largest Mediterranean island with a biodiversity that surpasses most other Mediterranean islands of similar or larger size, including a plethora of herpetofauna species inhabiting its terrain. In recent years, new roads were constructed on Lesvos, which are considered to be one of the most important factors that negatively affect the island's wildlife as they introduce disturbances [78], increase the non-natural mortality of animals, are obstacles to their mobility, and reduce the connectivity of populations by limiting their dynamics.

In order to provide an understanding of the impacts of road accidents on the herpetofauna species of the island and to contribute to the development of effective conservation strategies for these species, we identified the herpetofauna species most susceptible to road accidents and their spatiotemporal distribution patterns. We recorded roadkill data during the time periods of 2009–2012 and 2016–2019, and also analyzed the characteristics of the island's road network, aiming to address the following: (a) to determine the most frequently roadkilled herpetofauna species along the road network on the island of Lesvos, (b) to examine their temporal and seasonal patterns, (c) to evaluate the relationship between roadside habitats and road network characteristics in relation to these species, and (d) to identify their spatial patterns.

2. Materials and Methods

2.1. Study Area

Lesvos, the third-largest island of Greece and the eighth-largest of the Mediterranean basin with an area of 1632.8 km^2 and a population of approximately 90,000 inhabitants, is located in the northeastern Aegean Sea. The island is semi-mountainous with two high peaks (Mt. Lepetymnos and Mt. Olympus) and is covered mainly by traditional olive groves (*Olea europaea*) [79] in the eastern and southern part, continuous and homogeneous pine forests (*Pinus brutia* and *Pinus nigra*) in the central part, and brushwood vegetation along with scattered broadleaved forests (mainly *Quercus coccifera* and *Quercus ithaburensis*) in the north and western part (Figure 1). This landscape diversity, which is the result of the island's isolation, large size, complex geological history, and geographical position, makes Lesvos one of the most crucial wildlife zones in Europe with a higher biodiversity than most other Mediterranean islands of a similar or larger size, and a natural laboratory [80] promoting the existence of a high number of biota, including 30 herpetofauna species [81–83]. The island experiences a typical Mediterranean climate with pronounced spatial and seasonal fluctuations, marked by cool and moist winters and warm and dry summers [84], with annual rainfall varying from 677 mm in the eastern part to 415 mm in the western part [85].

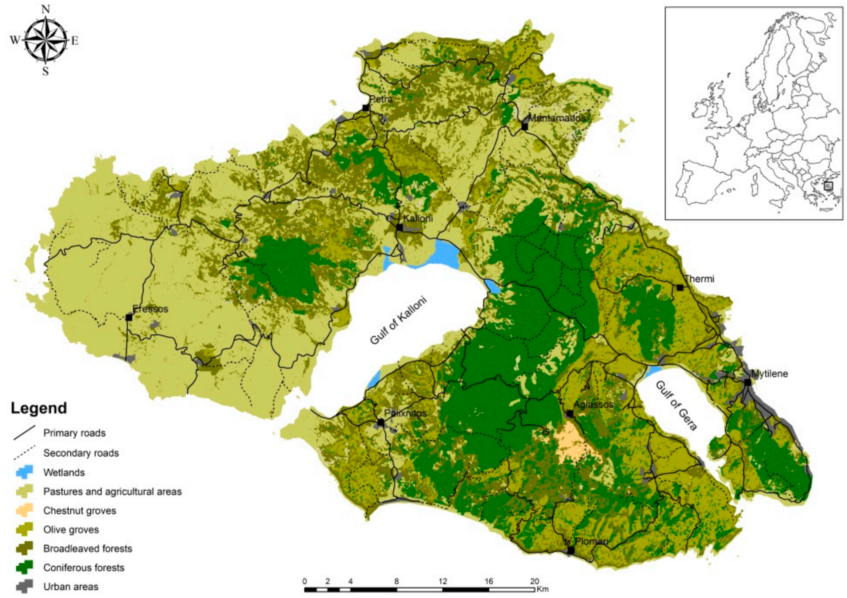

Figure 1. Map of the study area on the island of Lesvos, Greece, displaying the distribution of the main land habitats and the surveyed road network.

2.2. Identification of Road Network Characteristics and Mapping

To comprehensively gather all observations of herpetofauna species impacted by road mortality on the island of Lesvos, it was necessary to establish an accurate and complete representation of the road network present on the island. This was accomplished through the utilization of both open-source geospatial data from the Greek platform for geospatial data and services Geodata.gov.gr (accessed on 10 December 2022) and proprietary data from the Biodiversity Conservation Laboratory at the University of the Aegean. The main road network on the island spans 488.67 km and comprises primary roads connecting the island's capital, Mytilene, with smaller towns, while the secondary network spanning 484.18 km links towns to villages, and includes tertiary and dirt roads (Figure 1). Moreover, the primary road network consists of two lanes of traffic flowing in opposite directions and is equipped with an asphalt hard shoulder with an average width of 6.5 m, while the secondary network has an average width of 5 m. In parallel, it was imperative to document the vehicular density and velocity across the entire road network of Lesvos. As there was no available raw data, we made estimations by combining empirical observations with data obtained from relevant transport authorities and law enforcement agencies. Using this information, we characterized the roads into distinct categories: high (>100 vehicles/h), medium (50–100 vehicles/h), and low (<50 vehicles/h) vehicular density, and similarly, we classified the vehicle speeds as very high (>100 km/h), high (80–100 km/h), medium (50–80 km/h), and low (<50 km/h).

2.3. Road Mortality Survey

We conducted the road network surveys by driving a vehicle, using flashing emergency lights, at a low speed of 40–50 km/h (according to the minimum speed limits imposed by Greek legislation), along the roadsides. Our team of at least two experienced observers performed the field surveys during daylight hours. Over the course of two distinct time periods, from 2009–2012 and from 2016–2019, we executed a total of 96 surveys (12 per year) through consecutive surveys over multiple days each month to ensure thorough coverage of the entire road network. In parallel, we initiated monitoring at 07:00 to detect a maximum number of roadkilled herpetofauna carcasses from the previous night, thereby averting their destruction by vehicular traffic or scavengers. Monitoring ended at approximately 15:00. Cognizant of the tendency for numerous herpetofauna species to bask along the roadsides during the late afternoon and early evening [86], we prolonged the monitoring period in each survey from 17:00 to 19:00 h. To optimize the chances of finding all roadkills, monitoring was conducted in an alternating direction pattern, whereby the animals were only counted during transit. We meticulously recorded the GPS coordinates, date, and species of each roadkill observation, as well as the main roadside habitat and the road width, and we consistently removed any observed carcasses during subsequent surveys to eliminate the possibility of double-counting or additional roadkills of opportunistic predators. However, when the removal of roadkill carcasses was precluded by safety considerations, we exercised due diligence to prevent instances of double counting. The identification of the roadkilled herpetofauna was performed in the field with a high degree of specificity at the lowest taxonomic level during each survey.

2.4. Hotspot Analysis of the Roadkilled Herpetofauna

In order to analyze the clustering of reptile and amphibian road mortalities, we employed several spatial statistical tools within a GIS environment. To identify roadkill hotspots, we utilized two widely-used quantitative techniques: kernel density estimation (KDE) and the Getis-Ord Gi*.

We initially preprocessed our data using the Integrate and Collect Events tools in ArcGIS 10.7 Toolbox (ESRI Inc., Redlands, CA, USA) to aggregate the roadkill events in order to create a new weighted point feature class called ICOUNT, which served as the input for the hotspot analysis. Given the high incidence of closely spaced recorded events, the "Integrate and Collect Events" tools were utilized to consolidate features that are closely situated and generate a new feature class that comprises a single point at each distinct location, annotated with the relevant count, to signify the frequency of events [87]. We used the KDE to analyze the data at a broad scale, while the Getis-Ord Gi* method was utilized to examine the data on a local level, as it is particularly useful for analyzing local hotspots, allowing the identification of significant clusters at different scales and across different types of spatial relationships [88–90]. To ensure the accuracy of the analysis, we employed the Copy Features tool to preserve the original data and the Integrate tool to snap features together within a distance of 500 m, which was subjectively chosen based on our knowledge of the data and the road network of the island.

We employed the KDE, a non-parametric statistical method, to compute the concentration of roadkills around each cell of the output raster, by constructing a smooth curve, referred to as the "kernel," centered at each data point, which we summed to attain an estimate of the overall density of our data. KDE employs a kernel function to assign greater weight to data points that are closer to the point of interest, and a lower weight to those that are further away, to produce a smoothed density estimate [91]. We utilized this estimate to distinguish areas of greater or lesser roadkill density on a map, with the surface value peaking at the point location and diminishing as the distance from the point increases until it reaches zero at the search radius (bandwidth). Although KDE estimation creates a visually interpretable map, it does not provide markers of statistical significance such as z-scores and p-values [92]. To validate the results of the kernel density analysis at a local level, we proceeded to use the Getis-Ord Gi* analysis.

We used the Getis-Ord Gi* to identify regions where the mortality rate of reptiles and amphibians surpasses what would be expected by chance. Using this analysis, we calculated the roadkills' spatial autocorrelation in order to assess the similarity of the values between different locations. The Mapping Clusters tool, accessible in the ArcGIS spatial statistics tools suite, was utilized to conduct our analysis. The output of this tool comprised the GiZScore maps that indicated roadkill hotspots and coldspots by producing z-scores and p-values, which we used to determine the significance of the roadkills' clustering [92]. The direction and strength of the clustering are indicated by the z-score, while the likelihood of the clustering being a chance occurrence is indicated by the p-value. The strength of the clustering of high values increases in proportion to the magnitude of statistically significant positive z-scores, where a larger z-score implies a more pronounced clustering (designated as a hotspot). Conversely, the intensity of clustering of low values is inversely proportional to the magnitude of statistically significant negative z-scores, such that a smaller z-score signifies a more intense clustering (designated as a coldspot) [88]. Finally, we generated a multitude of maps to depict the results of the aforementioned analyses, both for the entire dataset and its various subcategories, including (a) the two examined time periods, (b) all reptiles and amphibians, (c) all snakes and lizards, (d) all seasons, and (e) the species with the highest mortality rate.

2.5. Statistical Analysis

All statistical analyses were conducted using SPSS software (v.25.0. IBM Corp., Armonk, NY, USA). To ensure the control of systematic error, it was imperative to carefully evaluate potential sources of variation, such as disparities in sampling methods or data collection procedures, before consolidating data from distinct time periods. In all of our surveys, we employed uniform techniques, protocols, and field researchers to gather primary data. Furthermore, prior to conducting additional analysis, we tested for possible differences in roadside habitats, vehicular density, vehicle speed, road category, and road width between the two time periods to determine whether pooling the raw data was appropriate. Categorical variables (roadside habitats, vehicular density, vehicle speed, and road category) were examined using chi-square tests, while a t-test was used to analyze the continuous variable (road width). Our results revealed no statistically significant differences between the two time periods (roadside habitats: $\chi^2 = 6.947$, df = 5, $p = 0.225$; vehicular density: $\chi^2 = 5.906$, df = 2, $p = 0.052$; vehicle speed: $\chi^2 = 7.251$, df = 3, $p = 0.064$; road category: $\chi^2 = 3.259$, df = 1, $p = 0.071$; road width: t = 1.920, df = 328, $p = 0.056$), and thus, we decided to pool the data.

We initially estimated a relative road mortality index (RMI) by dividing the number of roadkills for a particular species by the total number of roadkills for all species; this was used as an index of the relative frequency of roadkill events for each species [93]. In order to determine whether there was a significant association between roadkill events (number of casualties) per taxonomic group (reptiles and amphibians) and the four herpetofauna categories (amphibians, lizards, snakes, turtles) in relation to the following factors: (1) monthly and seasonal variations, (2) roadside habitats, including pastures and agricultural areas, olive groves, maquis vegetation, pine forests, wetland vegetation, and urban areas, and (3) vehicular density, vehicle speed, road category, and road width, we performed a set of chi-square tests. Owing to the limited sample size of the turtles' category (Table 1), we opted to exclude them from the chi-square analysis, thereby restricting our focus solely to amphibians, snakes, and lizards.

We also developed a binary logistic regression model (BLR), which analyzed several influential factors such as months and seasons, roadside habitat, vehicular density, vehicle speed, and road category and width, on roadkills of reptiles and amphibians by computing a classification table of observed and predicted values. Further, we evaluated the model's performance using receiver operating characteristic (ROC) curve analysis. The predictor variables were optimized through a backward stepwise procedure, and the model's overall significance was determined by the Hosmer–Lemeshow goodness of fit test. Additionally, we employed Nagelkerke's R^2 as an index to gauge the models' explanatory capability for the variation.

Table 1. Checklist of herpetofauna species recorded as roadkills during monitoring surveys on Lesvos. The number of roadkills regarding each species and the Road Mortality Index (RMI) is provided, along with the Annexes of Council Directive 92/43/EEC and the IUCN Categories corresponding to each species.

Species	Common Name	Roadkills (*n*)	RMI	92/43/EEC	IUCN
AMPHIBIA		130			
Anura					
Bufonidae					
Bufotes viridis	Green Toad	115	0.348	IV	LC
Hylidae					
Hyla orientalis	Eastern tree frog	2	0.006		
Ranidae					
Pelophylax bedriagae	Levant water frog	10	0.030	V	LC
Pelobatidae					
Pelobates syriacus	Eastern spadefoot	3	0.009	IV	LC
REPTILIA		200			
Testudines					
Emydidae					
Emys obricularis	European pond terrapin	1	0.003	II, IV	NT
Geoemydidae					
Mauremys rivulata	Western Caspian terrapin	4	0.012	II, IV	LC
Testudinidae					
Testudo graeca	Greek tortoise	2	0.006	II, IV	VU
Squamata					
Agamidae					
Laudakia stellio	Roughtail rock agama	3	0.009	IV	LC
Gekkonidae					
Hemidactylus turcicus	Mediterranean house gecko	2	0.006		LC
Lacertidae					
Lacerta diplochondrodes	Rhodos green lizard	40	0.121	IV	LC
Ophisops elegans	Snake-eyed lizard	31	0.093	IV	LC
Anguidae					
Pseudopus apodus	European glass lizard	54	0.163	IV	LC
Colubridae					
Dolichophis caspius	Caspian whipsnake	27	0.081	IV	LC
Telescopus fallax	European cat snake	3	0.009	IV	LC
Platyceps najadum	Dahl's whipsnake	2	0.006	IV	LC
Eirenis modestus	Ringheaded dwarf snake	2	0.006	IV	LC
Zamenis situla	European ratsnake	1	0.003	II, IV	LC
Natrix natrix	Grass snake	15	0.045		LC
Psammophiidae					
Malpolon insignitus	Eastern Montpellier snake	8	0.024		LC
Viperidae					
Montivipera xanthina	Ottoman viper	5	0.015	IV	LC
Total road mortalities		330			

3. Results

3.1. Herpetofauna Road Mortality

We documented 330 instances of road mortality among 20 species of herpetofauna, which included 130 amphibians and 200 reptiles, during our observational span of 2009–2012 and 2016–2019, on the road network of Lesvos (Figure 2).

All of the recorded roadkills were precisely identified to the species level. Our data was composed of four species of amphibians, three species of turtles, five species of lizards, and eight species of snakes, as elucidated in Table 1. A substantial proportion of the recorded species is classified as Least Concern (LC) by the International Union for Conservation of Nature (IUCN), with the exception of the Greek tortoise (*Testudo graeca*), which is classified as Vulnerable (VU) and *Emys obricularis*, which is classified as Near Threatened (NT). Of the recorded road mortality incidents, four species were identified as being the most impacted:

the green toad (*n* = 115, RMI = 0.348), the European glass lizard (*n* = 54, RMI = 0.163), the Rhodos green lizard (*n* = 40, RMI = 0.121), and the snake-eyed lizard (*n* = 31, RMI = 0.093) (Table 1).

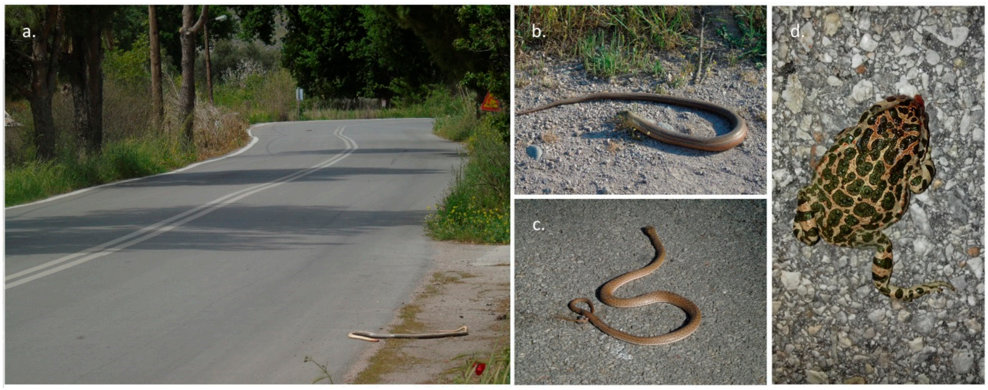

Figure 2. Roadkilled herpetofauna species in the road network of Lesvos: (**a**) *Dolchopis caspius*, (**b**) *Pseudopus apodus*, (**c**) *Malpolon insignitus*, and (**d**) *Bufotes viridis*.

Amphibians and lizards emerged as the herpetofauna categories with the highest incidence, each constituting 39.4% of the total road mortality events recorded. Snakes accounted for 19.1% of the incidents, while turtles accounted for only a mere 2.1%. In the amphibian category, the Green toad exhibited the most incidents, comprising 88.5% of the total amphibian road fatalities documented. Of the three species of terrapins present in Lesvos [83], the European pond terrapin (*Emys orbicularis*) and the Western Caspian terrapin (*Mauremys rivulata*) along with the terrestrial Greek tortoise were recorded in the turtle category. The Western Caspian terrapin accounted for the largest number of incidents (*n* = 4). In the category of lizards, the European glass lizard recorded the maximum number of incidents, making up 41.5% of the total, followed by the Rhodos green lizard at 30.8% and the snake-eyed lizard at 23.8%. Among the snake species, the Caspian whipsnake (*Dolichophis caspius*), found in many locations on the island, exhibited the largest percentage of incidents, constituting 42.9% of the total snake road fatalities recorded. Over the course of the first monitoring period (2009–2012), we recorded a cumulative 129 roadkill events, consisting of 62 amphibians and 67 reptiles, whereas during the second period (2016–2019), we documented a total of 201 incidents, comprising 73 amphibians and 128 reptiles. Moreover, a substantial number of incidents took place in roadside environments characterized by the prevalence of agricultural crops, olive groves, and maquis vegetation (Figure 3). In parallel, most of the observed roadside habitats are adjacent to freshwater bodies (small ponds, waterholes, channels, streams, and other freshwater marshes), which are abundant on the island [83].

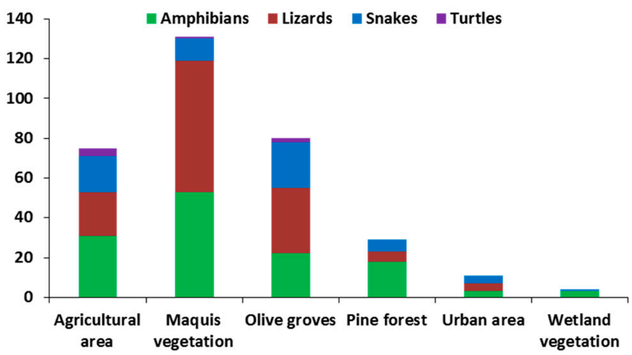

Figure 3. Distribution of road mortality among the four herpetofauna categories by roadside habitat.

3.2. Temporal and Seasonal Patterns of Roadkills

The majority of the roadkill events (190 out of 330) were observed to occur during the spring season, specifically in the months of April and May, constituting a substantial portion (57.6%) of the overall records (Figure 4). Among the categories of herpetofauna affected by road mortality, lizards emerged as the most affected group during this period, with a proportion of 49.5% of the total roadkill incidents (Figure 4), where the European glass lizard was the most recorded species, accounting for 24.2% of the incidents. The summer months exhibited the lowest number of roadkill incidents with a total of 39 events. Nevertheless, summer was the season with the highest incidence of snake mortality, accounting for 35.9% of the total, with the Caspian whipsnake having the highest number of records at 10.3%. During autumn, the category of lizards again recorded the highest number of roadkill incidents, with snakes exhibiting a relatively high proportion (33.3%), making them the second most affected group. Conversely, during the winter months (Figure 4), both snakes and lizards showed a low number of roadkill incidents due to the prevalent low temperatures during this period, where they were not active. On the other hand, amphibians emerged as the species with the highest mortality rate in winter, accounting for 95.2% of the total roadkill incidents, with 62 records in total.

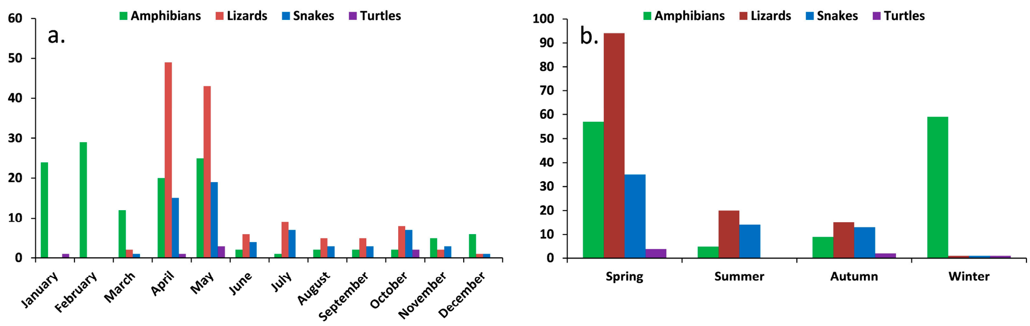

Figure 4. Distribution of herpetofauna road mortality incidents across (**a**) months and (**b**) seasons during both time periods.

The results of the chi-square analysis indicated a statistically significant discrepancy in the distribution of roadkill events between the reptile and amphibian taxonomic groups, as well as across the four herpetofauna categories, after excluding the turtles' category, with variations observed in both months and seasons (reptiles and amphibians (months: $\chi^2 = 125.45$, df = 11, $p < 0.0001$; seasons: $\chi^2 = 103.37$, df = 3, $p < 0.0001$); herpetofauna categories (months: $\chi^2 = 136.24$, df = 22, $p < 0.001$; seasons: $\chi^2 = 110.91$, df = 6, $p < 0.0001$)). The impact of roadside habitats and vehicular density on the incidence of roadkills among reptiles and amphibians was deemed statistically significant (roadside habitats: $\chi^2 = 15.12$, df = 5, $p < 0.001$; vehicular density: $\chi^2 = 12.35$, df = 2, $p = 0.002$). Conversely, there was no significant effect of vehicle speed ($p = 0.093$), road category ($p = 0.657$), or road width ($p = 0.493$) on the distribution of roadkills among these taxonomic groups. With regard to the distinct herpetofauna categories, the chi-square analysis revealed a significant impact of roadside habitats ($\chi^2 = 33.79$, df = 10, $p < 0.0001$) and vehicular density ($\chi^2 = 17.95$, df = 4, $p < 0.001$) on the distribution of roadkill incidents, while no disparities were detected with regard to vehicle speed ($p = 0.319$), road category ($p = 0.201$), and road width ($p = 0.250$).

3.3. Predicting the Occurrence of Reptile and Amphibian Roadkills

In order to ascertain the influence of temporal and seasonal factors, roadside habitat, vehicular density, vehicle speed, road category, and road width on the prevalence of reptile and amphibian roadkills, we adopted a Binary Logistic Regression (BLR) analytical approach. Our findings reveal that the month, roadside habitat, vehicular density, and vehicle speed have the most substantial effect on the prediction of the presence of roadkilled reptiles and amphibians (Table 2). The Nagelkerke R^2 demonstrated that these variables accounted for 60.3% of the total variance observed in the data. Additionally, the Hosmer–Lemeshow test confirmed the model's fitness, as the absence of significant chi-square values attested to its acceptability (Hosmer–Lemeshow = 9.057; $p = 0.338$). The area under the receiver operating characteristic curve (AUC = 0.893; SE = 0.01; 95% CI 0.857–0.928; $p = 0.0001$; Figure A1) was used to gauge the sensitivity of specificity values and, with a threshold of 0.604, correctly classified 81.8% of reptiles and amphibians with a predicted classification accuracy of 77% for roadkilled amphibians and 85.1% for roadkilled reptiles (χ^2 (22, N = 330) = 195.57; $p < 0.0001$; Table 2).

Table 2. Logistic regression model for the presence probability of reptiles and amphibians. The model log likelihood is a metric of the suitability of the model's fit, and the alteration in the −2 log likelihood gauges the enhancement in fit when compared to a more basic model. The degrees of freedom (df) are utilized to compute the *p*-value, and the significance of the log likelihood modification. The *p*-value embodies the sig. of the change, signifying the level of significance of the shift in the −2 log likelihood.

Predictor	Model Log Likelihood	Change in −2 Log Likelihood	df	*p*-Value
Month	−215.29	179.66	11	0.001
Roadside habitats	−134.71	18.49	5	0.002
Vehicular density	−136.10	21.27	2	0.001
Vehicle speed	−129.79	8.663	3	0.034
Road width	−131.18	11.44	1	0.001

3.4. Spatial Distribution and Hotspot Areas for Roadkilled Reptiles and Amphibians

The results of the KDE analysis revealed clusters of roadkilled herpetofauna species along the roadsides, as seen in Figure 5. These clusters were identified as areas with high-density values, signifying a greater concentration of roadkilled reptiles and amphibians compared to their surrounding areas. The Getis Ord Gi* analysis corresponded to these hotspots, which are areas of high vehicular density and speed.

The majority of these hotspots exhibit z-scores ranging from 2.11–3.11 and are situated in the northeastern region of the island near Mantamados, as well as in the western part near Eressos. Other notable hotspots were identified in the Kalloni area, exhibiting z-scores ranging from 0.91 to 2.10. These hotspots were characterized by a concentration of roadkilled reptiles and amphibians that exceeded chance and posed the highest risk for roadkill. Conversely, the most prominent coldspots are distributed throughout the northwestern and southeastern regions of the island. These results affirm the consistency between the hotspots identified by the KDE and those established through the Getis Ord Gi* analysis. The z-scores obtained from the Gi* statistic, illustrated as red dots for hotspots and blue dots for coldspots in Figure 5d, demonstrate the statistical significance of the identified clusters.

With regard to the two temporal intervals we analyzed, the hotspot analysis depicted a differentiation in the spatial distribution of roadkills. During the initial time frame of investigation (2009–2012), the hotspots were dispersed in the central and eastern regions of the island, with z-scores ranging from 1.27 to 2.20, as illustrated in Figure 6a,b. Conversely, during the second time frame (2016–2019), the hotspots were primarily concentrated in the central region near Kalloni, in the northeastern region near Mantamados, as well as in the western part of the island (Figure 6c,d).

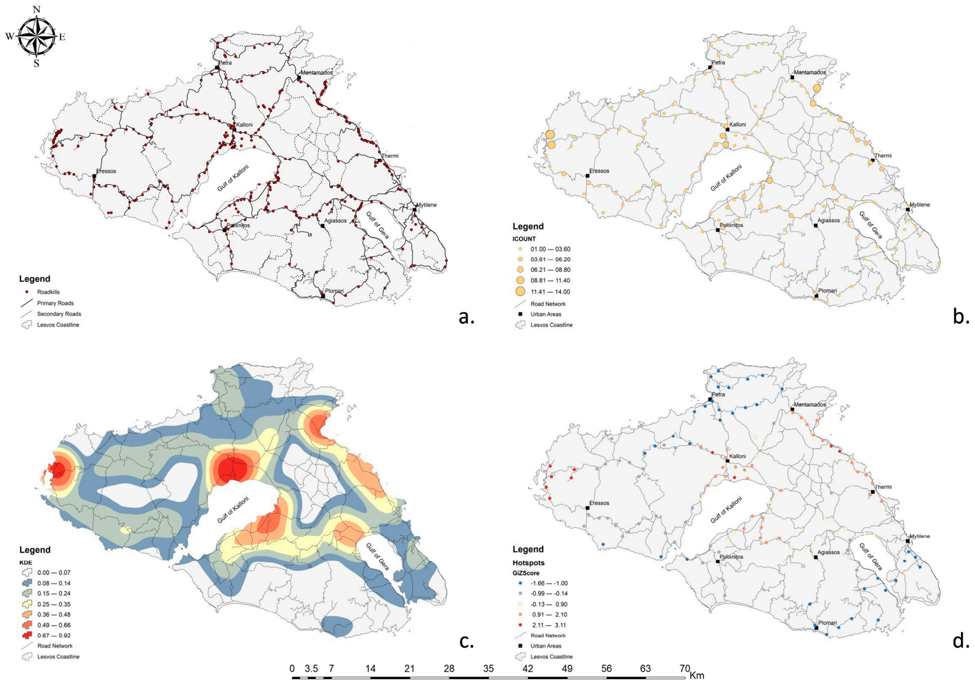

Figure 5. Procedure for the spatial analysis performed in ArcToolbox comprising several stages: (**a**) the geospatial representation of the road mortality incidents of herpetofauna species across the island, (**b**) the outcome of the Collect Events analysis, (**c**) the visual representation of the distribution of roadkilled reptiles and amphibians through kernel density estimation (KDE), and (**d**) the identification of the hotspots.

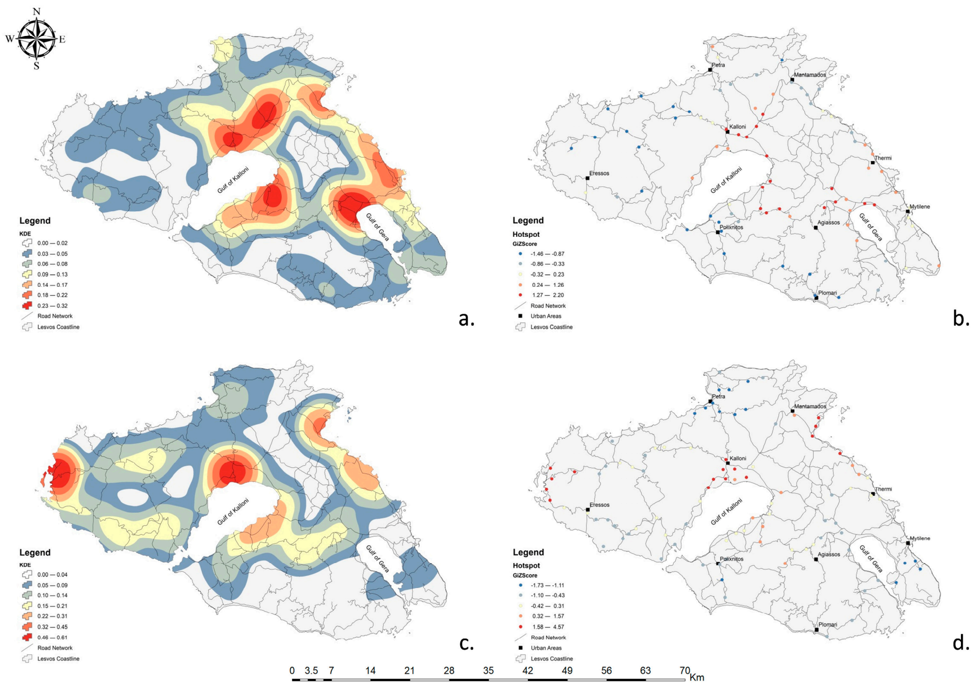

Figure 6. Hotspot analysis incorporating the kernel density estimation and the Getis Ord Gi*, presenting the distribution of roadkill hotspots in (**a**,**b**) the timeframe of 2009–2012, and (**c**,**d**) the period of 2016–2019.

The z-scores in Figure 7 reflect the direction and intensity of the clustering of roadkills in relation to their distribution across the seasons. Simultaneously, there is a pronounced differentiation in their spatial distribution, which is apparent as a consequence of the species ecological characteristics.

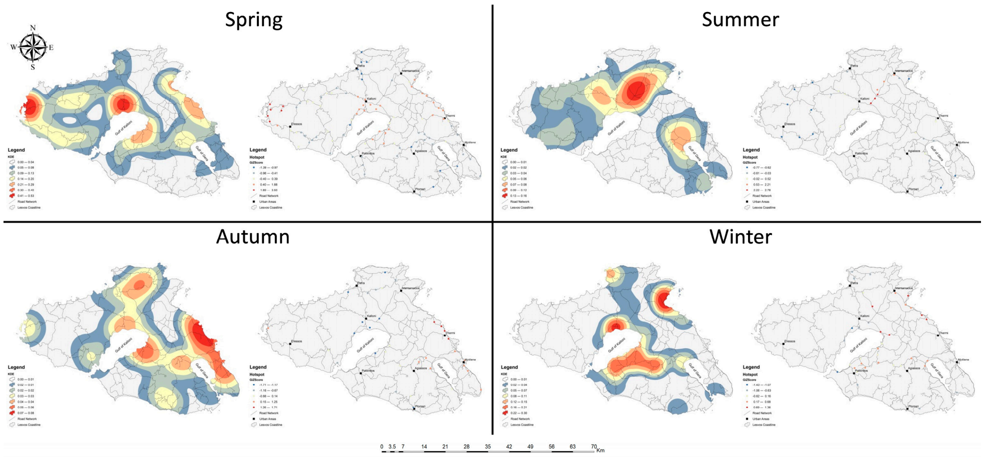

Figure 7. Seasonal distribution of the roadkill hotspots depicted through KDE and Getis Ord Gi*.

The analysis of amphibians and reptiles as taxonomic groups revealed pronounced variations in both the spatial distribution and degree of intensity. The hotspots of road mortalities among amphibians were observed to be situated near wetlands, freshwater marshes, and waterholes [83]. On the other hand, reptiles tend to experience a higher incidence of road mortalities in regions characterized by a dominance of agricultural land and pastures (Figure 8).

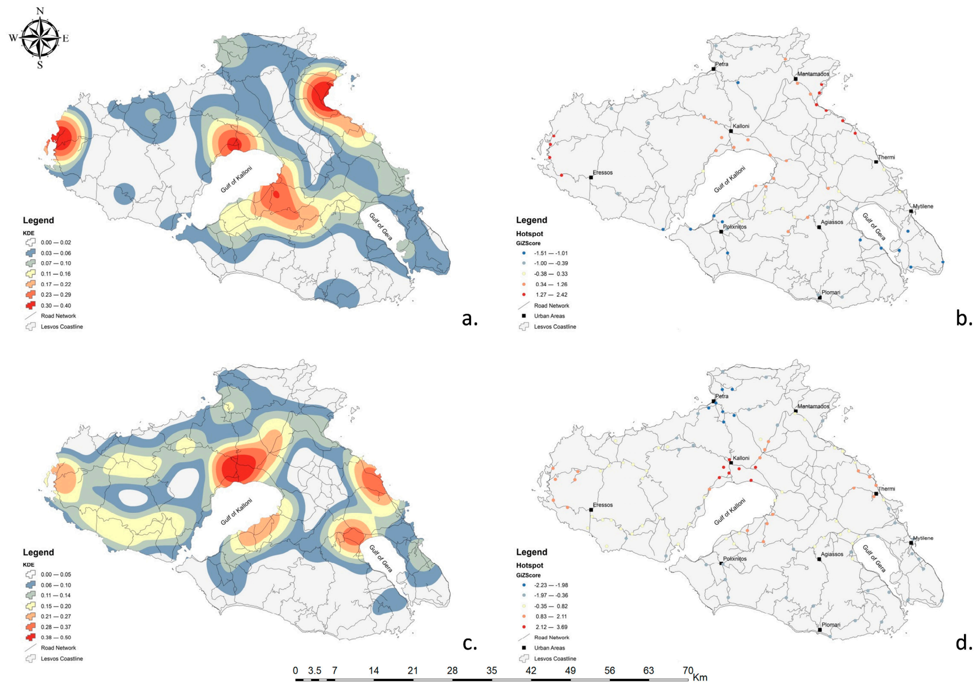

Figure 8. Hotspots of roadkills showcased via KDE and Getis Ord Gi*: (**a**,**b**) amphibians; (**c**,**d**) reptiles.

Finally, the results of the analysis of the four categories of reptiles (amphibians, lizards, snakes, turtles) and the most commonly impacted species in terms of roadkills are displayed in Figures A2 and A3, respectively. These results are consistent with the general spatial distribution pattern of the respective taxonomic groups.

4. Discussion

Investigating and understanding the threats to island biodiversity is of critical importance, as limited land area and concentrated populations make it more feasible and effective to implement conservation efforts. This is particularly true for islands such as Lesvos, where the available habitat may be limited and herpetofauna populations are already at risk due to habitat degradation and the introduction of invasive predators [83,94–96]. The isolation of island populations can result in reduced genetic diversity and increased vulnerability to diseases, parasites, predators, and other disturbances [97–99]. Furthermore, the construction of roads and other infrastructures exacerbates this isolation, hindering the mobility of individuals and reducing the flow of genetic material [100]. The life history traits of island-dwelling animal populations, such as their size, isolation, and the characteristics of the "insular syndrome" [101,102], render them particularly susceptible to human impacts, which are further amplified by the low reproductive rates and high adult survival characteristic of the "insular syndrome" [103]. As a result, island populations are at a disadvantage in terms of conservation [104] and are more vulnerable to human impacts compared to their mainland counterparts [105].

As in other parts of Greece, the island of Lesvos has experienced a recent surge in road construction in the last decade, both through the restoration of the existing road network and the creation of new routes (after 2019). This has resulted in heightened traffic intensity and higher vehicular speeds, which, in conjunction with the fragmentation of habitats, has led to frequent roadkill events affecting herpetofauna, as well as mammals and other animal taxa [106–108]. Road rehabilitation has been proven to contribute to increased speeds, resulting in an increase in roadkills [28]. Considering that aspect, we aimed to assess the herpetofauna species most susceptible to road accidents, as well as to analyze their geographical, temporal, and seasonal distribution patterns, during the time frames of 2009–2012 and 2016–2019. Additionally, we sought to examine the most commonly impacted herpetofauna species along roadways, the relationship between roadside habitats and road network features with roadkilled herpetofauna species, and the spatial patterns of herpetofauna road mortality.

To the best of our knowledge, our study represents the first examination of the road mortality of amphibians and reptiles in Greece and the first such study to be conducted on a Greek island. However, our results should not be viewed as definitive evidence of species population endangerment, as no comprehensive population density assessment of reptiles and amphibians on Lesvos has been conducted. Despite deploying at least two observers and driving at low speeds to maximize roadkill detection, it is likely that some fatalities went unrecorded, leading to an underestimation of the actual herpetofauna casualties on Lesvos' road network, as a result of scavengers, delayed mortality of injured animals, and weather conditions. Thus, the 330 reptiles and amphibians that we recorded must be considered a conservative estimate of the number of roadkills that actually occurred. Although surveys conducted with higher intensity (such as every two days) would increase accuracy, especially for small animals that remain on roads only for a brief period [109,110], financial constraints made this unfeasible; this is the reason why we performed surveys over a 2–3 day period once per month. Despite these potential inaccuracies, the roadkill patterns of amphibians and reptiles, while not absolute, are considered a true reflection of the Lesvos road network's roadkill pattern.

On Lesvos, certain herpetofauna species seem to be more susceptible to road-related fatalities than others. In particular, amphibian species were found to be the most vulnerable to vehicular accidents on the roadways of Lesvos, with the green toad emerging as the species with the highest recorded mortality rate, both in the overall set of records (115 out of 330) and in the category of amphibians specifically (115 out of 130), a phenomenon that aligns with the well-established notion that amphibians are one of the most susceptible groups of animals to road fatalities [41,56,58]. The elevated roadkill mortality rates of amphibians that we observe is in alignment with prior studies, taking into account road surveys conducted at reduced vehicular speeds [30,55]. This vulnerability is quite common in amphibian populations, which are characterized by a high incidence of mortality, surpassing that of other herpetofauna species, due to the intricate interplay of population structure, habitat, and activity and habit patterns [111], and it has also been linked to the diminishment of their populations [112,113]. During the breeding season, the aquatic nature of these species requires them to abandon their terrestrial wintering habitats and seek out freshwater bodies for reproductive purposes, increasing the probability of road-related mortality as a result of vehicular collisions during the migration, and/or if a road interrupts their transition between habitats [114]. The green toad, in particular, prefers habitats with Mediterranean vegetation and shrubs and during the breeding season, it seeks out wetter habitats [115]. We demonstrated the direct impact of this behavior on the viability of the species, as the highest number of recorded accidents was observed during the period of the breeding season, from the last winter months when it begins, to the last spring months when it ends.

It should be noted that, in addition to the green toad, several other species of herpetofauna were found to exhibit relatively high levels of road mortality. The European glass lizard, the Rhodos green lizard, and the snake-eyed lizard had the most frequently recorded incidents of roadkill. This is a result of both morphological factors such as a longer body length in comparison to other vertically crossing road animals [63] and behavioral attributes, including short distance movement by small-sized individuals and the exploitation of pavement temperature for thermoregulation [63,116,117]. When deciding to cross the road, they choose to move vertically on the road surface, following a consistent movement [116,118], in contrast to amphibians, which tend to orient in a specific direction and move with a wider distribution of angles [116]. For turtles, road mortality presents a constraint on population growth, especially those possessing reproductive capability, as a result of reduced adult survival and reproductive rates due to their delayed sexual maturity [59].

We examined the seasonal and monthly effects in roadkills of reptiles and amphibians, as the movement patterns of many of these species are known to be highly seasonal [113,119]. We found peak occurrences in spring (April and May) due to the interplay of varying weather conditions and temperature fluctuations as well as species physiology [59]. The results of the chi-square analysis provided us with valuable insights into the factors influencing the distribution of roadkill events among the reptile and amphibian taxonomic groups, as well as across the different herpetofauna categories, revealing a statistically significant discrepancy in the distribution of roadkill incidents, with variations observable in both months and seasons. This has been previously associated with wildlife phenology, activity patterns, and local climatic conditions [55,120]. Amphibians had the largest proportion of winter roadkill records, accounting for 95.2%, while reptiles recorded a comparatively small proportion of 1.6% each. This disparity can be attributed to the low temperatures prevalent during winter, which restrict the activity of reptiles, which are ectothermic and require relatively warm temperatures to be active [121]. Conversely, the high humidity levels during winter are conducive to amphibian activity and further augmented by the breeding season, which begins in February on Lesvos (pers. obs.). The biology of squamates (lizards and snakes) is significantly influenced by ambient temperatures, and as the temperature gradually rises in spring, their activity intensifies [122]. This is evident in our research, where lizards recorded the highest density of roadkill incidents, accounting for 49.5%, while snakes recorded a high proportion of 18.4% during this period. Amphibian roadkill incidents also recorded a significant proportion of 30% during spring, reflecting their continued intense activity as some species continue to breed and tadpoles of certain species commence their metamorphosis and move to drier habitats [123]. Summer weather conditions are more favorable for lizard and snake species, as the elevated temperatures enable them to use the road surface for thermoregulation [57]. Regrettably, this often leads to negative consequences for the species, as they are killed by passing vehicles, as evidenced by the present research where lizard roadkill incidents accounted for 51.3% and snake incidents accounted for 35.9% during the summer months. In autumn, the road mortality incidents of lizards and snakes also remained at elevated levels.

The diverse terrain surrounding the roadways of Lesvos (Figure 1) provides a heterogeneous landscape, and due to the fact that olive groves account for approximately a quarter of the island's total area and 87.4% of its agricultural lands, we considered it appropriate to differentiate olive groves from other agricultural regions [79]. This heterogeneity is exemplified by the substantial occurrence of roadkills in the surrounding areas dominated by maquis vegetation, with olive groves and other agricultural lands presenting a secondary level of occurrence (Figure 3).

The prevalence of vehicular density, velocity, road category, and road width are critical in assessing the frequency of roadkills among herpetofauna species [43,124]. However, our findings indicate that, in the case of reptiles and amphibians, only vehicular density demonstrated a statistically significant influence on the distribution of roadkills, while vehicle speed, road category, and road width exhibited no substantial effect. The morphological variations in the primary road network, including frequent elevation differences, dense roadside vegetation, and continuous turns, as well as the meteorological conditions [125], reduce the driver's visibility ([126], pers. obs.), which exacerbates the occurrence of roadkills [127,128]. The island's secondary road network also presents challenges (pers. obs.), such as poor road quality with potholes and cracks, inadequate lighting, and poorly marked roads, which further increase the risk of roadkills. As for the separate herpetofauna categories, our results deviated slightly, with vehicular density exerting a significant impact on the distribution of roadkill incidents. The lack of a notable effect of vehicle speed (p = 0.319), road category (p = 0.201), and road width (p = 0.250) underlines the importance of factoring in the specificities of each species when evaluating the factors affecting roadkills.

Notably, the combined examination of the impact of temporal and seasonal factors, roadside habitat, vehicular density, vehicle speed, road category, and road width on the occurrence of roadkills among amphibians and reptiles indicated that a binary logistic regression model can predict such incidents. With a classification accuracy of 77% for roadkilled amphibians and 85.1% for roadkilled reptiles, and an explanatory power of 60.3%, the model suggests that the probability of roadkill prediction per taxonomic group can be accurately determined by month, roadside habitats, vehicular density, vehicle speed, and road width. This approach highlights the most significant factors contributing to roadkills and can aid in devising mitigation strategies aimed at reducing the number of roadkills.

The performed kernel density estimations illuminated the existence of clusters in the roadkill data; however, they could not definitively ascertain whether these clusters resulted from chance or a spatial process [129]. Simply visualizing patterns was not sufficient in determining the hot spots with certainty [130]. Instead, the Getis-Ord Gi* analysis was utilized to test the null hypothesis of spatial randomness, thereby providing information on areas of high and low concentration in the roadkill data. By integrating this method with complementary spatial statistical techniques such as KDE, a comprehensive understanding of the spatial distribution of roadkills can be gleaned, as they can be used as surrogates for one another, informing conservation and management strategies aimed at mitigating road mortality threats to amphibian and reptile species [131].

We identified several hotspots of roadkills among reptiles and amphibians on the island of Lesvos along both the primary and secondary road networks (Figure 5). In particular, the western part of the island near Eressos exhibited a concentration of roadkilled young amphibians of the green toad and the Eastern spadefoot (*Pelobates syriacus*), who had recently undergone metamorphosis and were dispersed from their aquatic habitats (pers. obs.). These observations further indicate that this movement, facilitated by their behavior, led them to be highly mobile at dusk, particularly during an hour after sunset (pers. obs.). Similarly, in the northeastern part of the island near Mantamados, we found several killed adults of the species *Bufotes viridis* during the mating season. This region in Lesvos is considered a critical habitat for the species, which is enclosed by extensive primary and secondary roadways experiencing high vehicular traffic. Conversely, other significant breeding grounds with limited roadways and a diminished amount of vehicle traffic have much fewer road-related fatalities of the species, indicating the substantial impact of roadways and vehicular traffic on the species' road mortality in Lesvos. In this area, their behavior was characterized by intense mobility to and from breeding sites, leading them to cross roads, thereby increasing the risk of roadkills (pers. obs.). Another hotspot was identified in the central part of the island in the Kalloni region, which appeared to have a higher intensity of roadkills throughout both periods of our surveys. This region contains the largest wetland on the island, surrounded by agricultural areas and numerous canals that attract herpetofauna species and increase the likelihood of roadkills ([132], pers. obs.). These observations are in line with the results of the hotspot analysis of the most frequently affected species (Figure 8a,b and Figure A3).

Regarding the snakes, their hotspots are situated in regions with higher moisture and vegetation, which they prefer, and these hotspots are often along heavily trafficked roads, particularly during the spring and summer months when tourism is at its peak (Figure A2). In contrast, lizards can be found in various habitats all over the island, leading to hotspots in many locations. The most significant hotspot is in the northern part of the Kalloni region (Figure A2), an area that encompasses a range of agricultural, natural, and wetland ecosystems and is home to nearly all of Lesvos' species of lizards (pers. obs.), while it is crossed by one of the island's major roads with heavy, high-speed traffic all year round.

With regard to the other three species that recorded the highest incidence of road mortality, *Pseudopus apodus* (Figure A3) exhibits elevated levels of mobility during the spring season (pers. obs.). The concentration of hotspots in the western part of the island can be attributed to the presence of favorable habitats for the species and high vehicular activity due to the influx of tourists and the movement of local residents. This species bears a close resemblance to a snake, which often prompts drivers to deliberately kill it on the road (pers. obs.). Additionally, its physical structure restricts its movement on asphalt surfaces ([133], pers. obs.), while its large size makes it easily distinguishable during road surveys and, intriguingly, its carcasses were noted to persist on the road for longer periods than other reptiles, possibly due to reduced scavenger interest. The Rhodos green lizard (Figure A3) frequently uses roads for thermoregulation and experiences high levels of mobility during the spring season. This species, also easily distinguishable due to its large size, it is found in areas with suitable habitats with large populations (pers. obs.). These hotspots are typically located near agricultural lands, in combination with natural habitats, and are subjected to high vehicular traffic, particularly along the Mytilini–Kalloni-Petra or Mytilini–Mantamados routes, where substantial residential populations reside. Finally, the snake-eyed lizard (Figure A3) experiences the highest percentage of road mortality during the spring and summer months, when temperatures are high and its activity levels are intense. The numerous hotspots reflect the widespread distribution of the species across the island as well as its substantial population size, as it is one of the most commonly occurring reptile species on Lesvos (pers. obs.). Within these hotspots, appropriate habitats for the species are abundant, especially in the western part and around the Kalloni region.

In terms of seasonal hotspot differentiation, the hotspot between Kalloni and Petra (Figure 7) reaches its peak in summer with the influx of tourists to Petra, while winter hotspots are focused on amphibians near major breeding grounds and major roadways with year-round traffic (pers. obs.). A different hotspot emerges near Eressos in spring; this is due to the increased vehicular traffic resulting from visitors. Winter and autumn hotspots are situated along roadways with heightened traffic throughout the year and both related to breeding seasons, with winter hotspots aligning with amphibian breeding areas and autumn hotspots primarily associated with newly hatched snakes and lizards (pers. obs.).

The recent expansion of linear infrastructure on Lesvos and other Greek islands highlights the need to evaluate the impact of roads and traffic on wildlife and ecosystems. Hotspots of roadkilled herpetofauna species must be identified to facilitate conservation and mitigation efforts [134]. Our study reveals varying cluster locations along the road network dependent on time periods, seasons, and taxa. To minimize the impact on wildlife, we advocate the implementation of management measures, including traffic speed reducers, signage at hotspots, and environmental education [52]. Locally, crossing structures such as underpasses and overpasses in areas of high structural connectivity, guided by drift fencing, are recommended [135]. At the landscape level, priority should be given to protecting habitat patches and restoring degraded areas to enhance habitat connectivity [136]. The impact of roadways on ecosystems is a growing area of concern for researchers. As infrastructure continues to expand to accommodate human mobility, it is imperative that the design and implementation of road networks be meticulously planned to minimize adverse effects on ecosystems.

5. Conclusions

In conclusion, our study provides insights into the road mortality of herpetofauna species on the Greek island of Lesvos. Our results suggest that amphibian species, especially the green toad, are highly susceptible to road-related fatalities, which is consistent with previous studies. The green toad's behavioral trait of seeking wetter habitats during the breeding season increases its risk of road-related mortality. We also found that other species of reptiles, such as the European glass lizard, the Rhodos green lizard, and the snake-eyed lizard, exhibit relatively high levels of road mortality. However, the results of the study were considered a conservative estimate of the actual number of roadkills, as some fatalities may have gone unrecorded due to the limitations of the survey method. Despite these limitations, the roadkill patterns of amphibians and reptiles were considered a true reflection of the road network on Lesvos. It is important to note that the hotspot analysis revealed specific areas along the island's road network where herpetofauna road mortality was concentrated. This information can be used as a valuable tool for policymakers and wildlife conservation organizations [137] to identify priority areas for conservation efforts and road management strategies aimed at reducing roadkill events. This can lead to greater awareness and the adoption of safe driving practices, such as reducing speed in areas where wildlife is known to be active and being alert for potential hazards on the road [138]. By reducing the frequency of roadkills, it is possible to mitigate the negative impact of road construction and traffic intensity on herpetofauna populations and habitats on Lesvos.

Author Contributions: Conceptualization, Y.G.Z. and A.C.; methodology, Y.G.Z., A.K. and A.C.; software, Y.G.Z. and A.K.; validation, Y.G.Z.; formal analysis, Y.G.Z. and A.K.; investigation, Y.G.Z. and A.C.; resources, Y.G.Z., A.K. and A.C.; data curation, Y.G.Z.; writing—original draft preparation, Y.G.Z.; writing—review and editing, Y.G.Z., A.K. and A.C.; visualization, Y.G.Z. and A.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

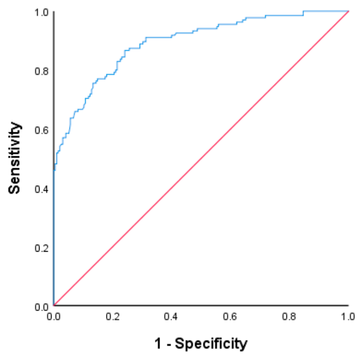

Figure A1. ROC curve for logistic regression model using the amphibians and reptiles dataset.

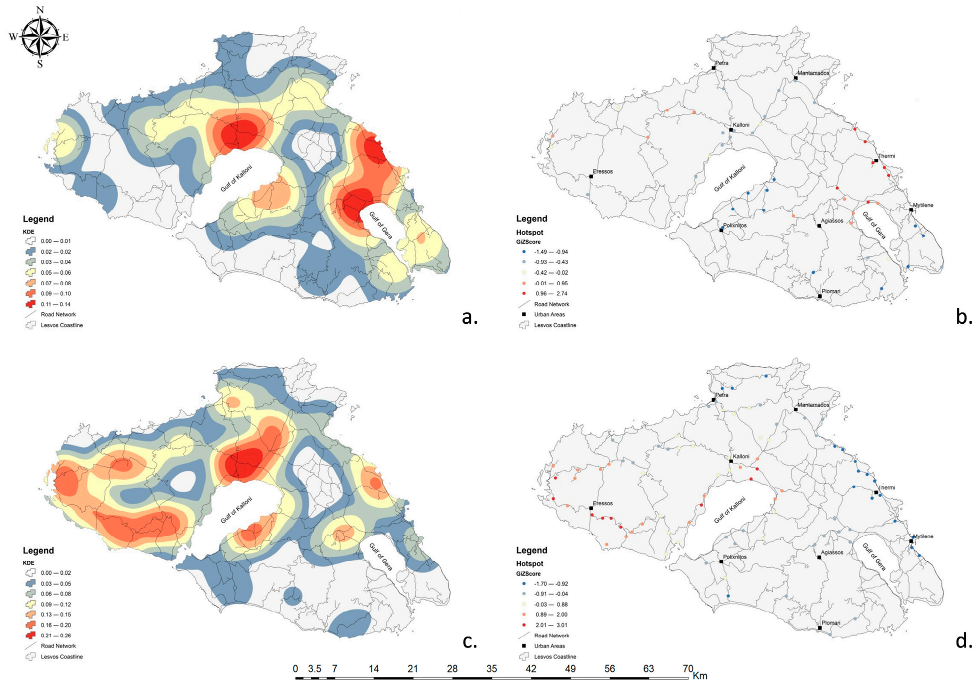

Figure A2. Hotspots of (**a**,**b**) snake and (**c**,**d**) lizard roadkills. It is noted that for the turtles' category, the analysis could not be performed due to the low number of roadkilled individuals.

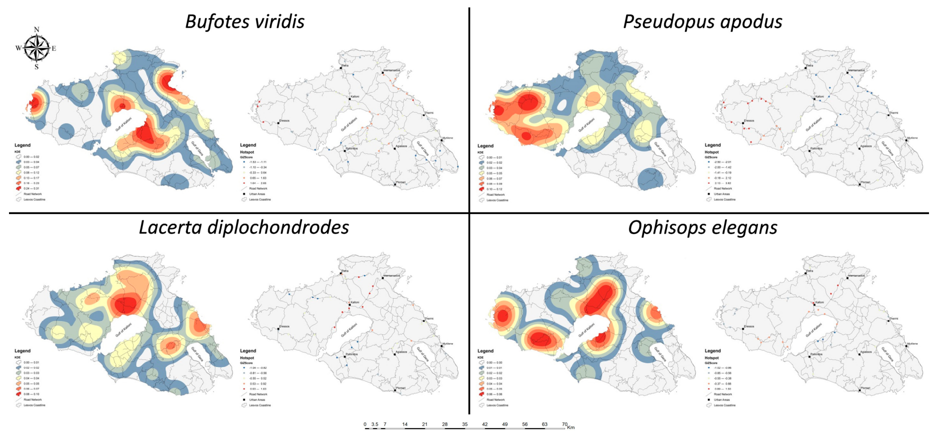

Figure A3. Hotspots of the top four roadkilled species.

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