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Wildlife Roadkill Driven by Hydrological Regime in a Subtropical Wetland

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Abstract: This study explored the role of the hydrological regime as a trigger factor for wildlife roadkill along a 22 km road crossing the Taim Wetland, a Ramsar site of international importance in South Brazil. The north–south crossing of BR-471, a federal highway, causes fatalities to numerous animals due to collisions with vehicles. An 11-year long-term time series study encompassing monthly roadkill incidents, water level, and rainfall was analyzed by considering three different periods related to a Wildlife Protection System (WPS): (i) 3 initial years before the WPS implementation (BWPS); (ii) 4 intermediate years after the WPS implementation (AWPS), which includes fences, fauna tunnels, cattle guard stocks, bumps, and speed control; (iii) 4 final years during partial destruction of the WPS (PDWPS). A pseudo-2D full hydrodynamic cell model was employed to fill missing water level data. The model had a good to very good performance (NSE: 0.73 to 0.87; R^2: 0.79 to 0.90). The relationship between roadkill incidents and the WPS periods (BWPS, AWPS, and PDWPS) was modeled using Generalized Additive Models for Location, Scale, and Shape (GAMLSS), considering rainfall and water level as predictor variables. The analysis revealed a higher incidence of wildlife roadkill in BWPS compared to AWPS and PDWPS, suggesting the effectiveness of the implemented measures. Critical season and interplay between water levels, rainfall, and the roadkill were assessed. Mammals was the most common roadkill class identified (~92%), followed by reptiles (13%) and birds (2%), with no change in these percentual in the BWPS, AWPS, and PDWPS. Among mammals, capybara (*Hydrochoerus hydrochaeris*) and coypu (*Myocastor coypu*) were the most frequent victims (~93% of mammals). Winter, followed by autumn, recorded the highest number of roadkill incidents (>60%), and this pattern remained consistent during the three periods. While rainfall did not emerge as a determining variable for roadkill, water levels above certain thresholds (>3.3 m) drastically diminished the effectiveness of the WPS, mainly due to fauna tunnel submersion. These findings offer valuable insights for enhancing wildlife conservation strategies in this protected area by incorporating hydrological information providing a baseline for designing WPS in similar environments.

Keywords: wetland; capybara; coypu; fauna tunnel; roadkill; wildlife protection system; water level

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1. Introduction

Although the invaluable ecosystem services delivered by wetlands have been recognized since 1971 in the scope of Ramsar Convention on Wetlands [1], coastal and inland wetlands across the globe continued to suffer from substantial loss and degradation [2–4]. An analysis of 189 reports detailing changes in wetland area reveals that the rate of wetland loss during the 20th and early 21st centuries has been almost four times faster than in previous centuries, with a staggering 64–71% of wetlands lost since 1900 [5]. Agricultural expansion (croplands, irrigation) and infrastructure development (e.g., roads), such as levees, water supply systems, and roads, have been pointed out as the main causes of wetland loss or degradation [4,6–11]

Roads are associated with economic and social benefits [12–17], facilitating transportation throughout floodplains and wetland areas, favoring regional development with positive effects normally proclaimed by policy makers and stakeholders [18–21]. However, the adverse environmental impacts of roads construction and operation are frequently downplayed [18,22–24]. These negative effects encompass habitat loss, landscape fragmentation, alterations in the physical and chemical environment, spread of exotic species [25–27], loss of biodiversity at both local and regional scales due to restricted movement between populations, increased human access to wildlife habitats, and intensification of bushmeat hunting [28–32]. In addition, some studies have revealed that road-induced isolation has led to reduced genetic diversity and increased genetic differentiation in snake populations (*Crotalus horridus*) due to interrupted seasonal migration [33].

Besides the environmental degradation, road infrastructure has been linked to increased mortality of wildlife due to earthmoving activities and collisions with vehicles [34,35]. Vehicle collisions with animals are major challenges [36], particularly when these accidents involve large animals [37,38], sometime resulting in human fatalities [39,40]. Some studies have demonstrated that the spatial distribution of roadkill along roads is not a random occurrence [41–45]. Instead, roadkill events tend to cluster along specific areas, particularly for large mammals and small-vertebrate fauna [46–48]. Consequently, various measures such as installing fences, implementing speed controls, employing wildlife warning signages, and constructing fauna tunnels are commonly employed strategies to mitigate the adverse impacts of roads on wildlife in these roadkill hotspots [49–55]. Moreover, roads adjacent to or crossing wetlands typically act as levees [30], necessitating drainage measures to stabilize roadbed embankments [56]. These measures can disrupt the natural hydrological regime [25,36,57–61], which plays a crucial role in shaping wetland characteristics [62–66]. Alterations in this natural variability can impact habitat suitability and trigger adaptive behavioral and spatial responses in various species [67–69].

Additionally, the natural water levels fluctuation due to weather conditions can alter both terrestrial and aquatic characteristics [25,70], affecting the persistence, distribution, and population dynamics of the resident species exposing them to different threats, including the roadkill [71–76]. When the cumulative stress from roadkill and hydrological changes surpasses certain thresholds, it may lead to reduced species numbers, biodiversity loss, or even long-term extinction [31,77–79], and mitigation measures to reduce roadkill can be only partly successful [36,55,60,61,80,81].

Despite extensive research on factors influencing roadkill rates, many studies offer short-term assessments spanning only a few months or years [82]. Few studies have evaluated the long-term effectiveness of mitigation measures, particularly through "before-and-after" analyses following the implementation of wildlife protection systems (WPS) [58,61]. In this study, we present an evaluation of an 11-year long-term time series of precipitation and water level from the Taim Wetland, a Ramsar site in South Brazil, combined with a simultaneous roadkill time series from a 22 km road (BR-471) that crosses this area from north–south. By considering a period before and after the implementation of a set of WPS, we evaluated the following: (i) the seasonal roadkill patterns; (ii) how the water level and rainfall triggers the animal movement increasing fatalities; and (iii) the feasibility of predicting monthly roadkill by using Generalized Additive Models for Location, Scale, and Shape (GAMLSS). Finally, we discuss the potential implications of our findings for enhancing the WPS.

2. Materials and Methods

2.1. Study Area

The study area is a Brazilian Federal Ecological Station (ESEC-Taim) which encompasses the Taim, a freshwater wetland assigned as the 2298 Ramsar site in 2017 [83]. ESEC-Taim spans 32,806 hectares and is located within the coastal plain of Rio Grande do Sul, the southernmost Brazilian State (Figure 1). In addition to the Taim wetland itself, the conservation area within the ESEC-Taim also comprises diverse ecosystems, including lagoons, grassy fields, dunes, and forests.

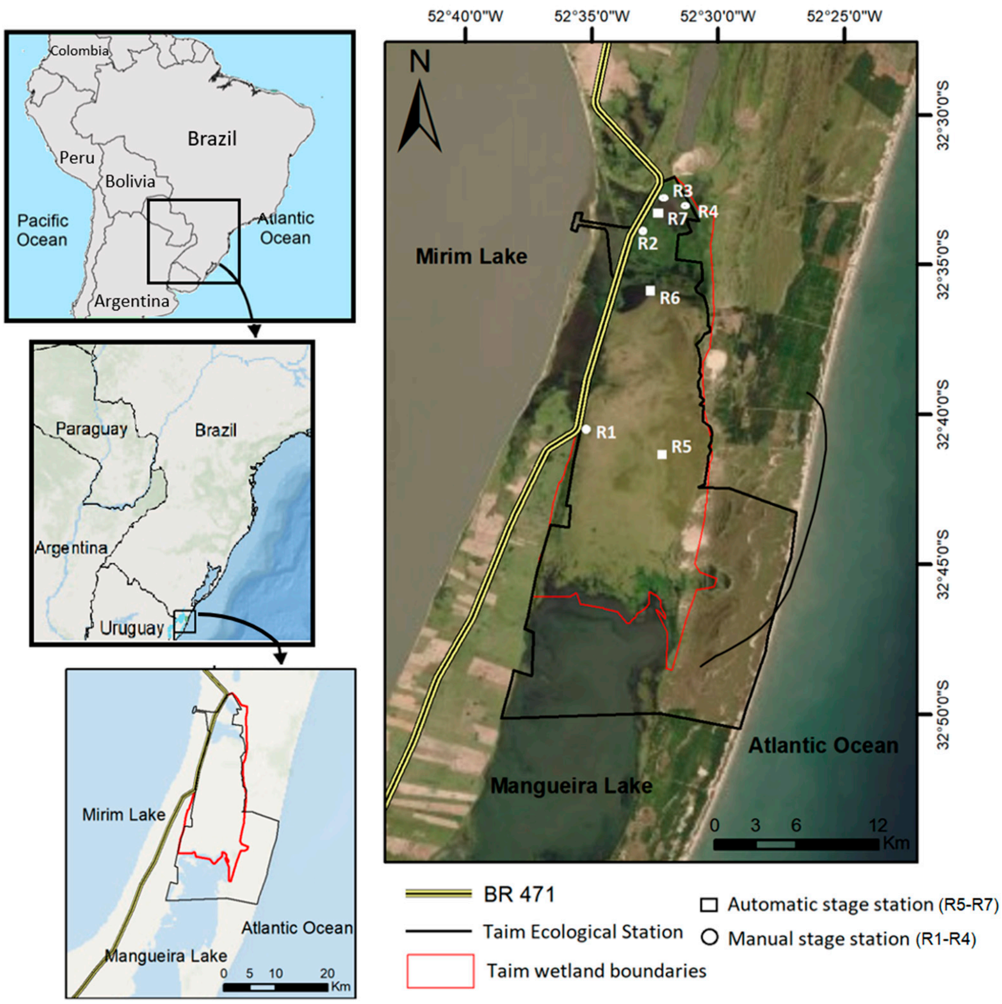


Figure 1. Study area, ESEC-Taim limits, and wetland location.

This site is one of the few remaining Brazilian wetlands with good conditions to harbor endangered species, notably the Atlantic yellow-nosed albatross (*Thalassarche chlororhynchos*), Flamarion's tuco-tuco (*Ctenomys flamarioni*), and the Neotropical otter (*Lontra longicaudis*). Furthermore, ESEC-Taim serves as a crucial transit point for migratory birds originating from the Northern Hemisphere, as well as those journeying from the Southern Cone of South America, such as the black-necked-swan (*Cygnus melancoryphus*), and others that contribute to the region's rich biodiversity.

Emergent aquatic macrophytes dominate extensive areas within the Taim wetland [84], providing a vital source of sustenance, shelter, and refuge for a diverse array of species. Moreover, the wetland plays a crucial role in shaping the hydrodynamics and carbon metabolism at its interface with the adjacent Mangueira Lake, where the roughness is increased, reducing water velocity, and consequently curbing the exchange of water and material between these water bodies [85].

Despite its significance, the Taim wetland shares its drainage basin with irrigated rice fields, from which substantial volumes of water (~100 $m^3 \cdot s^{-1}$) are withdrawn during the summer, coinciding with the peak of evapotranspiration in the Southern Hemisphere (October to March), leading to disruptions in the natural hydrological regime [78,86,87]. Consequently, this irrigation, coupled with the presence of road infrastructure, exerts significant pressure on the biodiversity within ESEC-Taim [83,86].

Road-related threats are due to the presence of BR-471, a federal single-lane road constructed in the 1960s to allow the post-harvest transportation of rice produced in that region (~30% of Brazilian rice production). This road was built through embankments bordering the Mirim lake floodplain (see Figure 1). It traverses approximately 22 km (from kilometer 491 to 513) of the western portion of the ESEC-Taim area, connecting the southernmost region of Brazil with Uruguay.

BR-471 acts as a levee, causing landscape fragmentation and diminishing both functional and structural connectivity between the wetland and the Mirim lake floodplain. Moreover, the road grants access for hunting, increases animal mortality due to vehicle collisions, and alters water hydrodynamics within the wetland. The road is largely regularly used by trucks for cattle transportation, but during the rice harvest season (March to May), the truck traffic increases substantially. These vehicles mostly ride at night when visibility is reduced and animals are most active, thus heightening the risk of roadkill incidents. Furthermore, during the austral summer, the traffic of Uruguayan tourists towards Brazilian beaches intensifies traffic, leading to increased fauna roadkill, mainly because BR-471 is a straight and flat road, which allows vehicles to reach high-speeds. Note that these roadkill incidents have significant impacts, affecting not only wildlife, including large mammals such as capybaras (*Hydrochoerus hydrochaeris*), but also leading to human fatalities [88]. Additionally, they contribute to environmental pollution resulting from accidents involving dangerous and toxic cargo as reported by ICMBIO technicians.

In response to a series of severe incidents, a Wildlife Protection System (WPS) was implemented along the ESEC-Taim in 1998 in an effort to mitigate roadkill issues. The WPS consists of 10.2 km long fences at 1.3 m height, cattle guards, and 19 fauna tunnels with a 1.6 m diameter (see Figure 2). These fences were installed along the northern (covering 3.4 km) and southern (spanning 6.8 km) segments of the road, positioned approximately 11 m from the edge of the lane. Cattle guards were placed at the beginning and end of the fenced sections, while fauna tunnels were distributed at one-kilometer intervals. Notably, the central road segment (covering 5.5 km), where the wetland exhibits greater connectivity with the Mirim lake floodplain, was left without fences; instead, road bumps and speed control measures, including one at km 500, were implemented. In May 2002, during extreme flooding, the WPS was partially destroyed, especially along 5 km of fences.

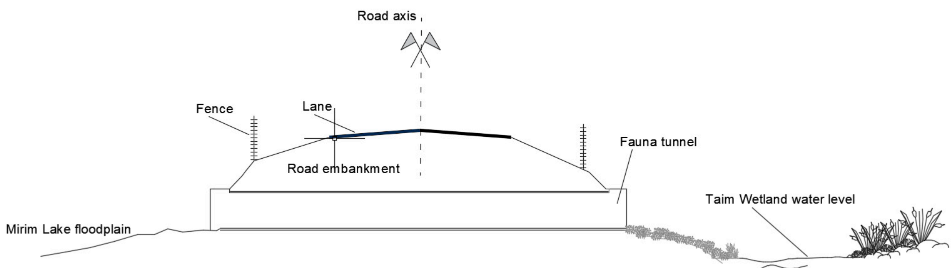


Figure 2. Schematic cross section of BR-471 crossing ESEC-Taim including the representation of fauna tunnel and fences.

A prior biological study compared species and roadkill rates before and after the WPS installation, using data spanning from 1995 to 2002 [89]. The results suggest potential inefficiencies in the WPS, as it primarily benefited one mammal species, the coypu (*Myocastor coypus*).

2.2. Roadkill Dataset Treatment

A roadkill time series spanning approximately 11 years (from July 1995 to April 2006) was used to assess the fauna roadkill incidents over the 22 km stretch of the BR-471 road adjacent to ESEC-Taim. The roadkill data gathering was performed daily during the morning by ICMBIO (Chico Mendes Institute for Biodiversity Conservation) technicians from the ESEC-Taim. The survey was accomplished by car, and any observed animal carcasses were promptly removed from the road to prevent recounts. The roadkill incidents involved mammals, birds, reptiles, and amphibians, and the recorded information included the date, month, year, species, and the specific kilometer at which the carcass was found.

To ensure data integrity, this time series was carefully checked for consistency, missing data, and potential errors during data recording. Overall, non-continuous data gaps (~11.5%) were especially associated with the vacation period of ESEC-Taim technicians or lack of financial resources to maintain the activity.

2.3. Hydrological Modeling and Dataset

The Taim Wetland lies within the international cross-border Mirim Lake basin, covering an approximate surface of 62,250 km^2. The Taim Wetland drainage basin can be divided into three hydrological subsystems: North, Wetland, and South [90]. The flow direction in the system is usually northward, from Mangueira Lake towards the Taim Wetland, and then from the wetland towards the Mirim Lake, with a possible flow inversion (backwater) under rare and specific conditions [91]. The hydrological supply to Taim Wetland primarily comes from direct precipitation and draining, notably from Mangueira Lake. The water loss from this system occurs due to direct evapotranspiration or drainage towards Mirim Lake [85], facilitated by a set of non-managed (permanently opened) man-made floodgates beneath the BR-471 road in the Northern part.

Rainfall has been monitored since 1940s through a network of rain gauges strategically positioned across the basin, ensuring a relatively good spatial distribution. Evaporation is systematically observed at two meteorological stations placed at the North and South extremes of the region. Although there are currently no flow measurements in the area, daily water level information was gathered from four manual stage stations (R1–R4) and three automatic ones (R5–R7), indicated in Figure 1. The water level time series from these stations mainly encompass a period from 1995 to 1996 and from 1998 to 2003. To align the roadkill and hydrological dataset spanning from July 1995 to April 2006, missing water level periods were filled using a pseudo-2D full hydrodynamic cell model tailored for the Taim Wetland [92], previously calibrated and validated [86,87,90,91].

The hydrological system in the model is delineated through two key modules:

(i) Basin Module: this module processes monthly rainfall-runoff in areas contributing to Mangueira Lake by employing a simple runoff coefficient formulation and calculates the water balance in the lake. Evapotranspiration and rainfall time series are derived using Inverse Distance Weighting (IDW) interpolation based on rain gauge data, presenting a monthly spatial representation of evapotranspiration and rainfall across the study area's watershed. This module provides an upstream boundary condition for the Wetland Module.

(ii) Wetland Module: this module operates as a cell-based hydrodynamic model [92,93], integrating the inflow from Mangueira Lake, the wetland hydrodynamics, and the influence of the Mirim Lake as the downstream boundary condition. The hydrodynamics within the wetland are ruled by a combination of factors, including topography, channels, and internal lagoons, besides the spatial distribution of emergent macrophytes which play a crucial role by introducing roughness to the flow. The model encapsulates this intricate and heterogeneous system by representing it through a series of irregular cells. The model also includes equations to represent the functioning of hydraulic structures, such as the floodgates and the fauna tunnels, which can act as a drainage pipe during submersion.

The combined functionality of the wetland module and the basin module comprehensively represents the entire system. For further insights into the model's intricacies and initial adjustments, refer to [86,87,92]. QGIS [94] was used for processing all spatial information. Model performance was assessed by comparing the observed and simulated water levels. The Nash–Sutcliffe coefficient (NSE) was used as the goodness-of-fit metric [95].

2.4. Data Analysis

Monthly roadkill data was assessed over the seasons, according to the WPS phase, which included the period before WPS (BWPS) from September 1995 until September 1998, after WPS (AWPS) from October 1998 to May 2002, and post-partial-destruction (PDWPS) from Jun 2002 to April 2006. For seasonality analysis, we considered the following seasons: summer (January to March), autumn (April to Jun), winter (July to September), and spring (October to December). This dataset was also examined concerning roadkill according to the classes of animals (mammals, birds, reptiles, and amphibians), as well as the total roadkill incidents. Because mammals, particularly capybaras and coypu, constituted most roadkill cases, the mammal category was analyzed with these two species individually, while other mammals were assessed separately. The group of other mammals includes species such as the Molina's hog-nosed skunk (*Conepatus chinga*), white-eared opossum (*Didelphis albiventris*), Pampas fox (*Dusicyon gymnocercus*), crab-eating fox (*Cerdocyon thous*), six-banded armadillo (*Euphractus sex-cinctus*), and crab-eating raccoon (*Procyon cancrivorus*).

Hydrological data were processed according to the season, months, long-term monthly average, frequency, and water level duration curve. This comprehensive approach allowed for an in-depth examination of their individual relationship with the recorded roadkill incidents. In cases where water level data were considered, only months with complete roadkill data were included in the analysis, avoiding uncertainties related to the roadkill amount. The long-term rainfall time series represents the monthly spatially averaged rainfall in the watershed.

The statistical analysis to evaluate the effectiveness of WPS in reducing roadkill counts over the seasons involved comparing BWPS, AWPS, and PDWPS. This comparison was conducted using the non-parametric Kruskal–Wallis test (KW) [96] with a post hoc Kruskal–Wallis test performed at a significance level of 5%. The statistical analyses were carried using the agricolae package [97] in R [98]. Correlations analyses were conducted in R [98], based on a process of rank comparison by determining the Spearman correlation coefficient with a minimum significance level of 5%.

Generalized Additive Models for Location, Scale, and Shape (GAMLSS) were used to model the relationship between roadkill over the periods analyzed (BWPS, AWPS, and PDWPS) and predictor variables of rainfall (mm) and water level (m). GAMLSS were chosen due to their ability to model not only the mean of the response variable (location) but also the variability (scale) and the shape of the distribution. GAMLSS are versatile tools for a variety of applications, from time series analysis to modeling count data [99–103], among others. GAMLSS modeling was accomplished using the gamlss package [104] in R [98].

The basic GAMLSS regression model can be expressed as follows [105]:

$$g_k(\theta_k) = \eta_k = X_k\beta_k + \sum_{j=1}^{J_k} Z_{jk}\gamma_{jk} \quad (1)$$

where $g_k(\cdot)$ is a link function for the kth parameter θ; k = 1, 2, . . ., K; η is the linear predictor; β is the fixed effect parameter vector of the linear model; X is an experimental matrix; γ is a vector of random effects associated with variables and/or factors expressed in the experimental matrix Z.

Due to the discrete nature of roadkill counts, regression models based on discrete probability distributions were employed. Additionally, only fixed effects ($X_k\beta_k$) were considered in the models. AIC and BIC criteria were considered to identify the best regression model. Regression models based on the Poisson Inverse Gaussian (PIG) and Double Poisson (DPO) were fitted to the dataset, depending on the analysis performed. Both PIG and DPO distributions involve determining two parameters (μ and σ). The μ parameter was modeled considering all covariate, while σ was modeled only by the intercept.

To assess model performance, a refined index of agreement (*d*) proposed by Willmott [106] was used to compare predicted roadkill with observed values. This index varies from 0 to 1, and 1 means a perfect fitting between the roadkill estimated by the model and the monitored value.

3. Results

3.1. Water Level Modeling

The water levels predicted by the 2D hydrological/hydraulic model demonstrated good to very good [107] performance at different stage stations used as control. In Figure 3, scatter plots for four stations within the Taim Wetland area shown along with the Nash–Sutcliffe (NSE) efficiency and R^2 values. The notably high NSE (from 0.73 to 0.87) and R^2 (from 0.79 to 0.90) values confirm the model's robust performance, effectively reproducing the water level dynamics.

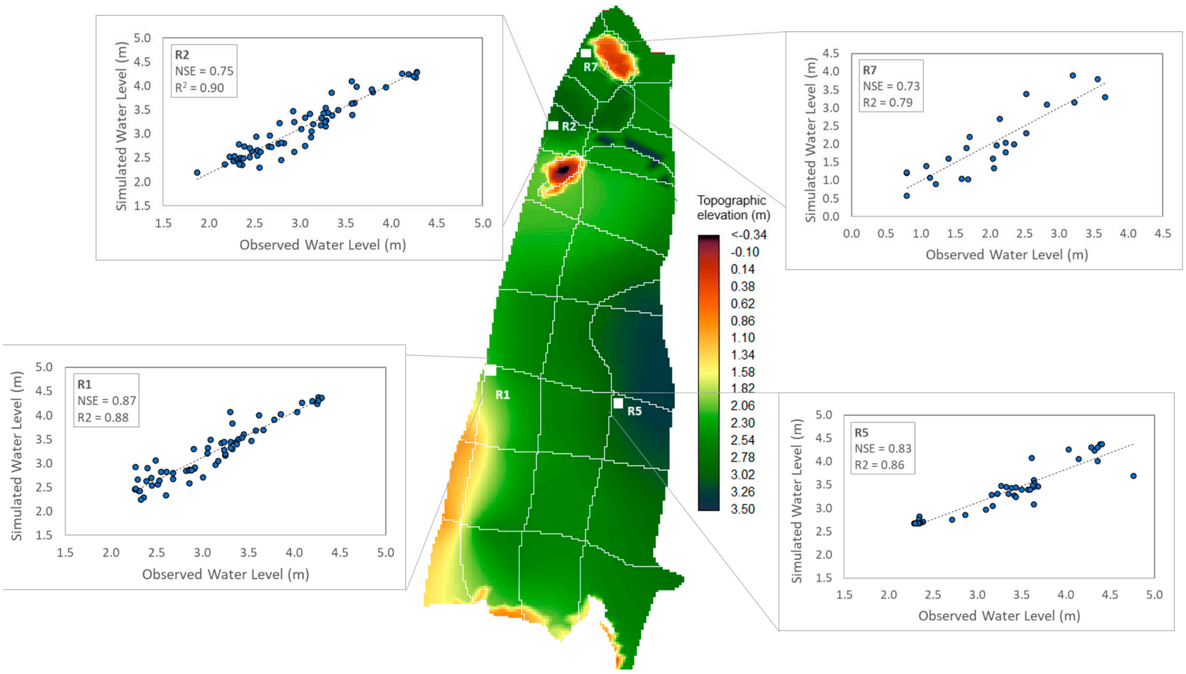
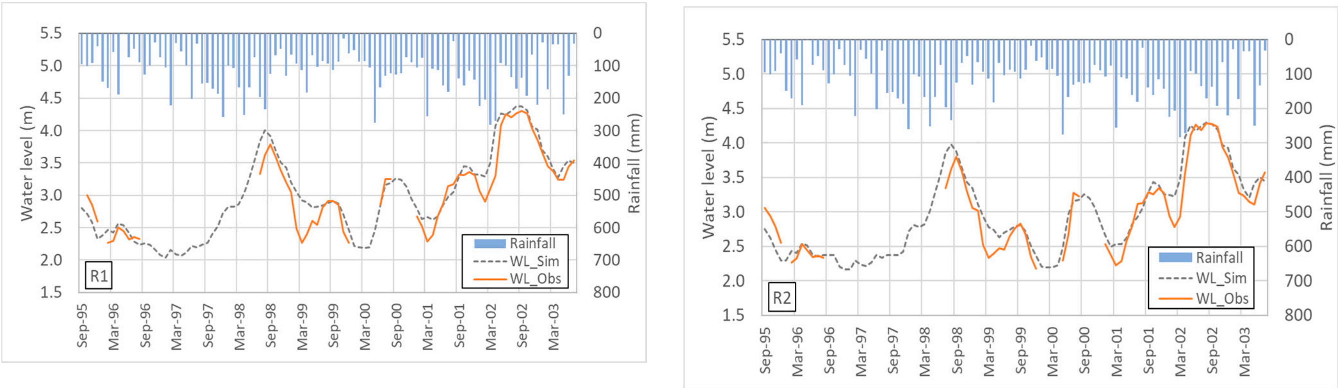


Figure 3. Scatter plots for the four representative water level stage stations within Taim Wetland—white lines in the Taim area indicate the cells of the model over the topographic elevation.

Noteworthy among these stations, R1, R2, and R7 (Figure 3) are strategically placed at the interface between the wetland and the road embankment. Figure 4a,b show observed and simulated water level at the R1 and R2 stage stations. These figures evidence how the water level dynamic was well represented throughout the period assessed, satisfactorily representing the low- and high-water-level periods.



(**a**) (**b**)

Figure 4. Observed (WL_Obs) and simulated (WL_Sim) water level at R1 (**a**) and R2 (**b**) stage stations within the Taim Wetland.

3.2. Roadkill Analysis

During the analyzed period from July 1995 to April 2006, roadkill data were collected along 3138 days, which accounts for approximately 79% of the total days within this timeframe. Throughout this period, a total of 3130 animal roadkill incidents were observed, indicating an average of nearly one roadkill per day. Figure 5 illustrates the long-term roadkill time series, displaying monthly totals according to the numbers of individuals separated in classes: mammals, birds, reptiles, and amphibians.

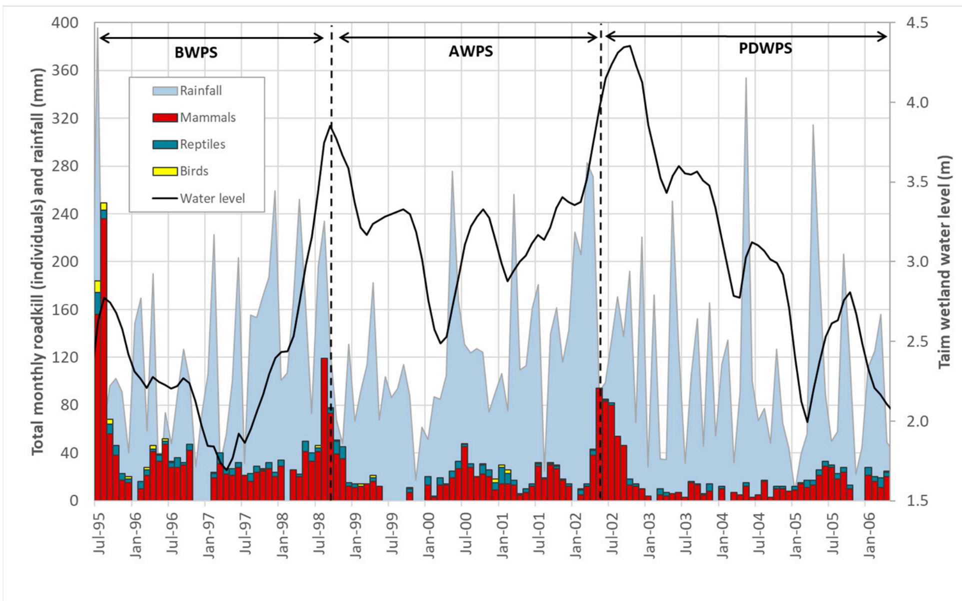


Figure 5. Long-term roadkill along BR-471 from July 1995 to April 2006, rainfall, and Taim wetland water level long-term time series.

Over the long-term period assessed, nearly 91.8% of roadkill incidents were associated with mammals. Among these mammal-related incidents, capybaras accounted for 47.9%, while coypus comprised 44.8%. The remaining 8.2% of roadkill incidents were attributed to reptiles (6.3%), including turtles, snakes, lizards, and others, while the remaining 1.9% were associated with birds. Amphibians were not recorded during this period.

There was a statistically significant difference (p-value < 0.05) in the number of roadkill incidents between the periods assessed (Table 1). The averages of mammals, reptiles, and total roadkill were statistically different when comparing the three periods assessed, as well as the average coypu roadkill. The average of birds roadkill during the BWPS and AWPS was statistically equivalent, but statistically different from the PDWPS period. Furthermore, the average roadkill of capybaras in the BWPS period was different from the AWPS and PDWPS periods, which were statistically equal. The same result was observed when assessing the group of other mammals roadkill.

Table 1. Average roadkill (individuals·month^{-1}) over BWPS, AWPS, and PDWPS periods separated by classes (birds, mammals, reptiles), total, capybara, coypu, and other mammals.

Period	Total	Mammals	Reptiles	Birds	Capybara	Coypu	Other Mammals
BWPS	45.2 a	42.2 a	5.0 a	1.1 a	16.2 a	23.0 a	3.0 a
AWPS	22.9 b	19.9 b	3.7 b	0.6 a	10.9 b	7.6 b	1.4 b
PDWPS	17.3 c	16.3 c	2.3 c	0.1 b	9.8 b	5.3 c	1.3 b
KW *p*-value	<0.0001	<0.0001	0.0004	0.0003	<0.0001	<0.0001	0.0014

Notes: averages followed by different lowercase letters denote significant differences among the periods period evaluated with the Kruskal–Wallis post hoc test (p-value < 0.05).

When the BWPS period is compared to the AWPS and PDWP periods, it is observed that the average monthly roadkill incidents decreased across all classes (Table 1), although the proportion of roadkill among the different classes remained relatively consistent (Figure 6). However, when the classes of mammals are analyzed (detail in Figure 6), an increase in the proportion of roadkill involving capybaras is observed after the installation of the WPS (BWPS = 38.4%; AWPS = 55.0%; PDWPS = 59.8%), coinciding with a decrease in the proportion of coypu roadkill (BWPS = 54.5%; AWPS = 38.0%; PDWPS = 32.3%). Notably, the most significant impact of the WPS installation was observed in coypu roadkill, with an absolute average reduction of approximately 17 individuals·month^{-1}, even after the partial destruction of the WPS in 2002.

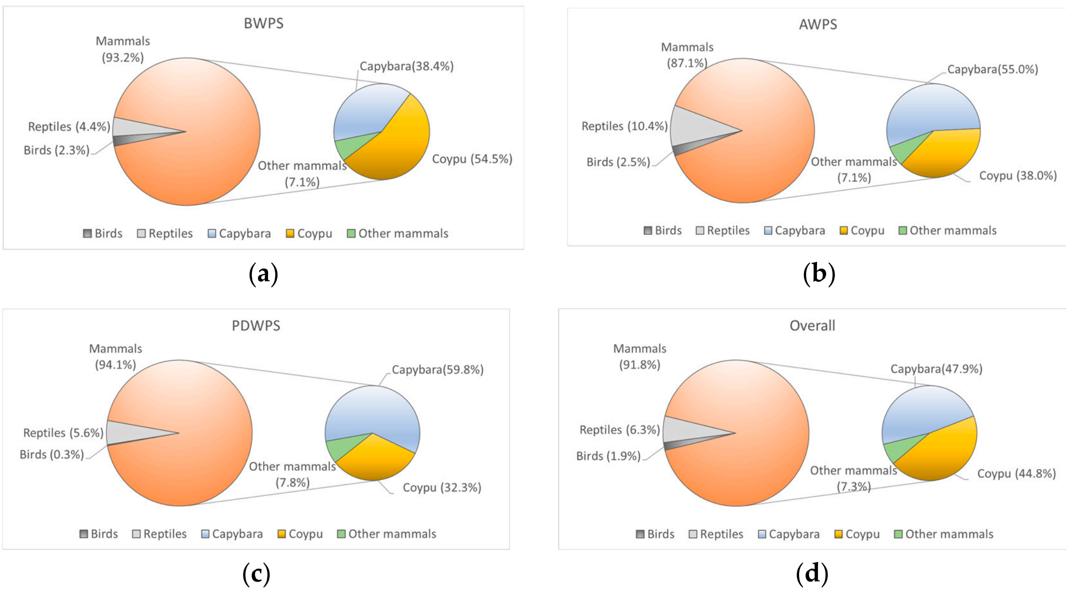


Figure 6. Roadkill proportion by classes for the (**a**) BWPS, (**b**) AWPS, (**c**) PDWPS, and (**d**) overall period, including details for mammals.

A long-term analysis in the database reveals that the rates of total roadkill, as well as roadkill related to the mammals class and capybaras roadkill, were statistically different throughout the seasons (p-value < 0.05), with the winter having the highest number of roadkill occurrences (Table 2). The roadkill rates for the reptiles and birds classes, coypu, and other mammals roadkill were statistically similar.

Table 2. Average roadkill (individuals·month^{-1}) across the seasons separated by classes (birds, mammals, reptiles), total, capybara, coypu, and other mammals.

Period	Total	Mammals	Reptiles	Birds	Capybara	Coypu	Other Mammals
Spring	20.7 b	17.4 bc	3.9 a	0.4 a	9.4 bc	7.1 a	1.0 a
Summer	16.3 b	13.6 c	4.3 a	0.4 a	7.4 c	4.2 a	2.0 a
Autumn	25.6 b	24.4 b	3.1 a	0.4 a	13.0 b	9.1 a	2.2 a
Winter	45.4 a	43.4 a	3.0 a	0.9 a	17.7 a	23.7 a	2.0 a
KW *p*-value	0.0040	0.0004	0.2330	0.8965	<0.0001	0.0882	0.1551

Notes: averages followed by different lowercase letters denote significant differences among the periods period evaluated with the Kruskal–Wallis post hoc test (*p*-value < 0.05).

When considering the influence of seasons on roadkill incidents along the three periods analyzed, it was observed that the highest levels of roadkill incidents are winter-related. During winter, the average roadkill incidents dropped from 72.8 individuals·month^{-1} in the BWPS period to 31.5 individuals·month^{-1} in the AWPS period. The tendency in the reduction of total roadkill in this season was also observed for the PDWPS period (25.6 individuals·month^{-1}), despite the damage to the WPS. No statistically significant difference was observed over the seasons for the BWPS and PDWPS periods (Table 3). However, during the AWPS period, there were statistically significant differences (*p*-value < 0.05) in the number of total roadkill, mammals roadkill, and specifically capybara roadkill across seasons. The amount of roadkill for the classes of reptiles and birds, as well as coypu and other mammals, was not statistically different across the seasons in the same period of analysis.

Table 3. Average roadkill (individuals·month^{-1}) across seasons by considering the BWPS, AWPS, and PDWPS periods.

Period	Season	Total	Mammals	Reptiles	Birds	Capybara	Coypu	Other Mammals
BWPS	Spring	29.9 a	26.3 a	4.6 a	0.4 a	14.7 a	10.1 a	1.4 a
	Summer	25.7 a	22.7 a	4.8 a	0.5 a	11.8 a	8.5 a	2.3 a
	Autumn	33.6 a	31.8 a	4.8 a	0.7 a	16.3 a	11.8 a	3.7 a
	Winter	72.8 a	69.0 a	5.5 a	2.0 a	19.2 a	46.1 a	3.8 a
	p-value *	0.2399	0.1069	0.9950	0.6199	0.0934	0.5584	0.2048
AWPS	Spring	21.0 ab	18.5 ab	5.2 a	0.7 a	10.3 b	8.6 a	1.1 a
	Summer	14.0 b	13.0 b	4.5 a	0.7 a	5.8 c	3.4 a	2.1 a
	Autumn	16.5 b	14.5 b	2.6 a	0.3 a	13.0 bc	9.7 a	1.6 a
	Winter	31.5 a	28.5 a	1.5 a	0.5 a	18.0 a	10.0 a	0.3 a
	p-value *	0.0415	0.0171	0.1028	0.6387	0.0043	0.1351	0.2759
PDWPS	Spring	10.2 a	8.6 a	2.1 a	0.0 a	4.7 a	3.4 a	0.5 a
	Summer	12.6 a	10.8 a	3.6 a	0.0 a	6.5 a	2.5 a	1.8 a
	Autumn	19.2 a	18.5 a	2.2 a	0.2 a	10.4 a	6.5 a	1.6 a
	Winter	25.6 a	25.4 a	1.3 a	0.0 a	16.2 a	8.1 a	1.2 a
	p-value *	0.3404	0.1992	0.2412	0.1136	0.0525	0.9591	0.2340

Notes: * KW *p*-value. Averages followed by different lowercase letters denote significant differences among the periods period evaluated with the post hoc Dunn's test (*p*-value < 0.05).

3.3. Combining Hydrological and Roadkill Analysis

Long-term monthly averages of water levels, rainfall, and the wildlife roadkill along BR-471 (Figure 7) were used to analyze monthly trends. Figure 7a vividly illustrates a distinct pattern accompanying the Taim Wetland hydroperiod: roadkill incidents exhibit a rapid increase during the rising limb of water levels, and subsequent decrease as water levels recede. Notably, this coincides with the autumn and winter season, corroborating the results presented in Table 2.

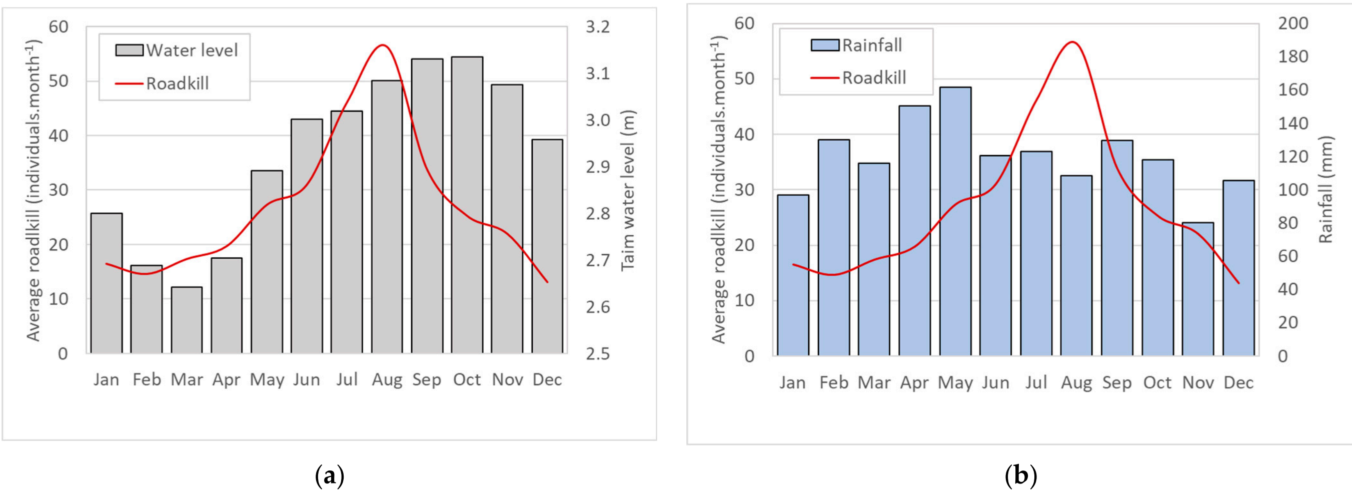
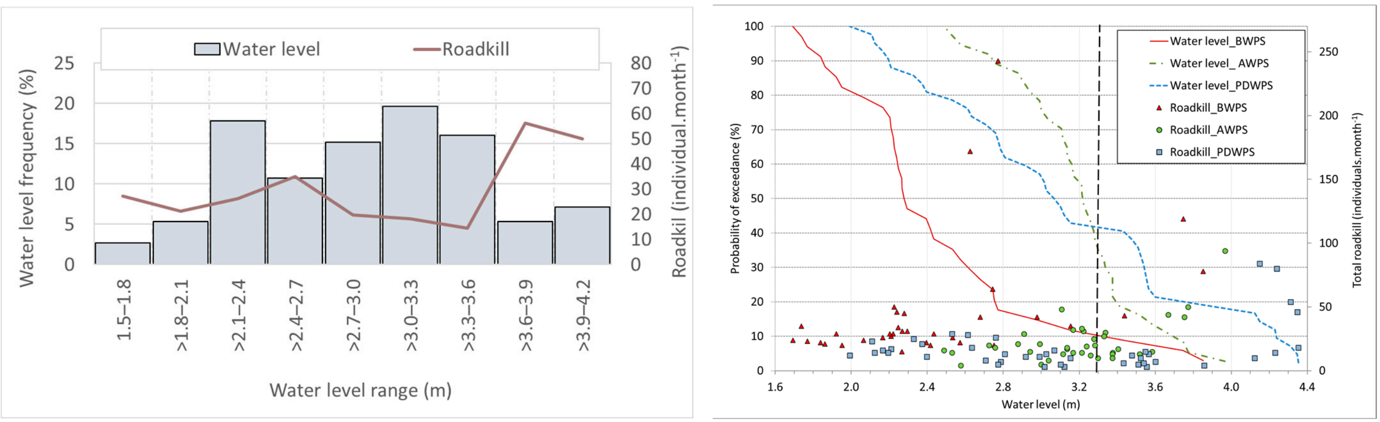


Figure 7. Long-term relationship between roadkill and Taim's monthly average water level (**a**) and monthly average rainfall (**b**).

The average monthly peak of roadkill (August) occurs approximately one month before the average water level peak in the Taim Wetland (September–October) (Figure 7a). In fact, a strong positive Spearman correlation (0.86) was found between the long-term average of monthly water level and the monthly roadkill incidents. To ascertain whether a specific water level threshold triggers an increased occurrence of wildlife roadkill incidents, a long-term water level frequency analysis was performed (Figure 8a) and assessed in combination with the roadkill data. This analysis showed that the average roadkill rate is approximately 26.7 individuals·month^{-1} when the water level is below 3.3 m. However, there is a significant spike in the average roadkill rate (40.2 individuals·month^{-1}) when the water level exceeds this threshold, with an approximate rate of 18.72 roadkill for every 10 cm of water level increase. The same frequency analysis was performed by segregating the water level time series into the BWPS, AWPS, and PDWPS periods, all leading to the same results. Notably, almost 35% of total roadkill occurred under these hydrological conditions (water level > 3.3 m) along the overall period. When considering the AWPS and PDWPS periods, almost 48% of the wildlife roadkill were monitored during water levels higher than 3.3 m.

Specifically, when analyzing the water level duration curve for the three periods assessed (Figure 8b), it is apparent that this condition was present only 10% of the time during the BWPS, 35% of the time during the AWPS, and 42% of the time during the PDWPS. For water level above 3.3 m, the proportion of roadkill was 15% (BWPS), 44% (AWPS), and 39% (PDWPS). The lower proportion of roadkill during the BWPS may biased by the presence of an outlier (243 roadkill during August 1995) for a water level below 3.3 m, as identified through the Grubbs' test. The same water level duration curve also allows us to identify that, for the same water level, the number of recorded roadkill incidents is generally lower during the AWPS and PWPS in comparison to the BWPS period, suggesting the of the WPS. Contrastingly, the direct relationship between monthly rainfall and roadkill incidents is less straightforward (Figure 7b).



(**a**) (**b**)

Figure 8. Average roadkill for each water level frequency range (**a**) and water level duration curves and total roadkill (**b**).

3.4. Roadkill Modeling

The long-term dataset underwent a comprehensive assessment using GAMLSS regression models, seeking to evaluate their ability to reproduce the observed collisions. A set of analyses was performed by considering the monthly data for wildlife roadkill, water level, and rainfall time series, as presented in the methodology. The Poisson Inverse Gaussian (PIG) produced the best fit for total roadkill, mammals, and birds classes (Table 4), as well as for capybara, coypu, and other mammals (Table 5). For reptiles roadkill, a model utilizing the Double Poisson (DPO) distribution was fitted, and the parameter estimates are presented in Table 6.

Table 4. Parameter estimates, standard error, *p*-value for total roadkill, and roadkill for the mammals and birds classes using the Poisson Inverse Gaussian model.

Roadkill Class/Group	Parameter	Estimate	Standard Error	*p*-Value
Total roadkill AIC = 922.11; BIC = 935.79		$\log(\mu)$		
	Intercept	2.81	0.30	<0.0001
	AWPS	−0.95	0.18	<0.0001
	PDWPS	−1.34	0.17	<0.0001
	Water level	0.41	0.11	0.0005
		$\log(\sigma)$		
	Intercept	−0.79	0.18	<0.0001
	Model equation	$\hat{\mu}_T = e^{(2.81-0.95\times AWPS-1.34\times PDWPS+0.41\times Water\ level)}$		
Mammals roadkill AIC = 905.84; BIC = 919.52		$\log(\mu)$		
	Intercept	2.69	0.32	<0.0001
	AWPS	−1.04	0.19	<0.0001
	PDWPS	−1.35	0.18	<0.0001
	Water level	0.42	0.12	0.0005
		$\log(\sigma)$		
	Intercept	−0.68	0.18	<0.0002
	Model equation	$\hat{\mu}_M = e^{(2.69-1.04\times AWPS-1.35\times PDWPS+0.42\times Water\ level)}$		

Table 4. *Cont.*

Roadkill Class/Group	Parameter	Estimate	Standard Error	*p*-Value
Birds roadkill AIC = 194.28 e BIC = 202.48.			$\log(\mu)$	
	Intercept	−0.22	0.23	0.3396
	PDWPS	−2.84	0.78	0.0004
			$\log(\sigma)$	
	Intercept	0.97	0.52	0.0654
	Model equation	$\hat{\mu}_B = e^{(-0.22-2.84\times PDWPS)}$		

Notes: $\hat{\mu}_T$ is the estimated average total roadkill (individuals·month^{-1}); $\hat{\mu}_M$ is the estimated average mammals roadkill (individuals·month^{-1}); $\hat{\mu}_B$ is the estimated average birds roadkill (individuals·month^{-1}); AWPS indicates the condition after WPS implementation, =0 when absent and =1 when present; PDWPS indicates the condition after the partial destruction of the WPS, =0 when not considered and =1 when considered; water level represents the average monthly water level in the Taim wetland (m).

Table 5. Parameter estimates, standard error, *p*-value for capybaras, coypus, and other mammals roadkill using the Poisson Inverse Gaussian model.

Roadkill Class/Group	Parameter	Estimate	Standard Error	*p*-Value
Capybara roadkill AIC = 756.89; BIC = 770.57			$\log(\mu)$	
	Intercept	2.13	0.29	<0.0001
	AWPS	−0.75	0.18	<0.0001
	PDWPS	−0.91	0.17	<0.0001
	Water level	0.31	0.11	0.0065
			$\log(\sigma)$	
	Intercept	−0.99	0.20	<0.0001
	Model equation	$\hat{\mu}_{CA} = e^{(2.13-0.75\times AWPS-0.91\times PDWPS+0.31\times Water\ level)}$		
Coypus roadkill AIC = 706.71; BIC = 720.39			$\log(\mu)$	
	Intercept	0.65	0.47	0.1730
	AWPS	−1.68	0.27	<0.0001
	PDWPS	−2.27	0.27	<0.0001
	Water level	0.94	0.18	<0.0001
			$\log(\sigma)$	
	Intercept	0.11	0.22	0.6380
	Model equation	$\hat{\mu}_{CO} = e^{(0.65-1.68\times AWPS-2.27\times PDWPS+0.94\times Water\ level)}$		
Other mammals roadkill AIC = 396.97; BIC = 405.18			$\log(\mu)$	
	Intercept	3.24	0.52	<0.0001
	Water level	−0.94	0.18	<0.0001
			$\log(\sigma)$	
	Intercept	−0.46	0.36	0.1940
	Model equation	$\hat{\mu}_{OM} = e^{(3.24-0.94\times Water\ level)}$		

Notes: $\hat{\mu}_{CA}$ is the estimated average capybara roadkill (individuals·month^{-1}); $\hat{\mu}_{CO}$ is the estimated average coypus roadkill (individuals·month^{-1}); $\hat{\mu}_{OM}$ is the estimated average of other mammals roadkill (individuals·month^{-1}); AWPS indicates the condition after WPS implementation, =0 when absent and =1 when present; PDWPS indicates the condition after the partial destruction of the WPS, =0 when not considered and =1 when considered; water level represents the average monthly water level in the Taim wetland (m).

Table 6. Parameter estimates, standard error, and p-value for roadkill of the reptiles class using the Double Poisson model.

Roadkill Class	Parameter	Estimate	Standard Error	*p*-Value
Reptiles roadkill AIC = 524.14; BIC = 535.09		$\log(\mu)$		
	Intercept	2.49	0.38	<0.0001
	PDWPS	−0.61	0.20	0.0034
	Water level	−0.37	0.14	0.0084
		$\log(\sigma)$		
	Intercept	0.94	0.16	<0.0001
	Model equation	$\hat{\mu}_R = e^{(2.49-0.61\times PDWPS-0.37\times Water\ level)}$		

Notes: $\hat{\mu}_R$ is the estimated average reptiles roadkill (individuals·month^{-1}); PDWPS indicates the condition after the partial destruction of the WPS, =0 when not considered and =1 when considered; water level represents the average monthly water level in the Taim wetland (m).

The GAMLSS regression models indicated a negative relationship between the AWPS period and roadkill, suggesting that the installation of WPS led to a reduction in roadkill incidents. Similar results were obtained for the PDWPS period, indicating that even with partial WPS destruction in 2002, there was still a decrease in the number of roadkill incidents. Conversely, there was a positive effect of Taim water levels, showing that an increase in water levels leads to an increase in the number of total roadkill incidents, including mammals class, capybaras and coypus; other mammals roadkill was affected just by the water level. These findings align with the previously presented water level frequency analyses, although this type of model does not allow for the identification of the specific water level threshold triggering an increase in roadkill incidents.

There was a negative relationship between the PDWPS and birds roadkill, and a negative relationship between the PDWPS and water level in reptiles roadkill. Additionally, it can be observed that the rainfall was not identified as a significant factor in the models, in accordance with previous analysis.

The efficiency index (d) (Table 7) used to assess the performance of the GAMLSS models adjusted indicates that, overall, models performed reasonably well (d > 0.5) in reproducing total roadkill incidents, as well as roadkill within the mammals class. It is noteworthy that the models also showed acceptable performance for capybaras, coypus, and the group of other mammals. However, when examining individual periods, some variations become evident. The model adjusted for capybaras performed well during the BWPS period but experienced a drop in performance in the last two periods. In contrast, the model adjusted for coypus performed well during the AWPS and PDWPS periods. Overall, the best results were achieved in modeling the group of other mammals during the PDWPS period (d > 0.75). The model developed for reptiles performed poorly, especially during the AWPS, while the model for birds showed the weakest results during the PDWPS period.

Table 7. Model performances, according to Refined Index of Agreement (d), for the 11-year time series (overall), and for BWPS, AWPS, and PDWPS periods.

Roadkill	Overall	BWPS	AWPS	PDWPS
Total	0.58	0.33	0.42	0.39
Mammals	0.57	0.32	0.36	0.37
Birds	0.34	0.14	0.30	0.00
Reptiles	0.34	0.41	0.18	0.38
Capybara	0.54	0.55	0.26	0.30
Coypu	0.55	0.39	0.53	0.55
Other mammals	0.53	0.28	0.32	0.75

4. Discussion

Wildlife roadkill along the stretch of BR 417 that crosses the Taim Wetland was evaluated by comparing a period with high incident levels to two subsequent periods after the installation of a wildlife protection system (WPS) composed of fences, fauna tunnels, cattle guard stocks, bumps, and speed control measures. The analysis showed that most roadkill along this segment involved mammals, particularly capybaras and coypus, regardless of the period assessed (before and after WPS). Furthermore, results demonstrated the effectiveness of WPS in reducing roadkill incidents, even after partial destruction of the system caused by heavy flooding in the region. Among the species evaluated, coypus appeared to be the biggest beneficiary of the wildlife protection system, agreeing with previous WPS effectiveness analysis [89] that considered roadkill counts until June 2002. Our results advance this analysis by considering a longer dataset and show that even with the partial destruction of the fences in 2002, the number of roadkill incidents was reduced in comparison to a period before the implementation of the WPS.

Seasonality is typically associated with the temporal patterns of roadkill incidents [34,35,75], and the roadkill rates along BR-471 exhibited a clear seasonal pattern across the assessed time periods. The different local species exhibit diverse responses to climate and weather conditions, leading to distinct seasonal activity patterns affecting the frequencies and roadkill rates. These patterns encompass movements, abundance, and the frequency of road crossings, thus influencing roadkill incidents [108]. The effects of climate and weather conditions on roadkill rates have been frequently considered in studies aiming to understand this intricate relationship. Different investigations have shown that migratory, mating, breeding, rutting, and hunting seasons are highly correlated to roadkill incidents [109–112]. Favorable climates can encourage high levels of species abundance and richness within habitat patches [113], thereby contributing to a higher occurrence of roadkill incidents when these species are active near or on the road [113,114]. Conversely, some research suggest that adverse conditions may elevate the probability of incidents [115,116], while other studies argue that such conditions can discourage the movement of certain animals, reducing roadkill incidents [117].

Higher rates of mammals roadkill, especially during winter, corroborate findings from previous studies in the same region [89,118]. Our findings indicate that the peak of mammals roadkill during the winter precedes the peak of water levels in the Taim Wetland, in alignment with field observations by ESEC-Taim technicians, who report that rising water levels lead to heightened animal movements, particularly among mammal species, resulting in an elevated roadkill risk during this period. These valuable field observations not only validate the qualitative findings presented here but also underline the importance of considering hydrological elements when implementing roadkill mitigation strategies and road safety measures. Analogous behavior was observed for a South Florida road, whose characteristics are like BR-471, in which the roadkill were mainly related to the availability of standing water in roadside ditches and pasture wetland [119]. These results can be possible explained by the seasonal aggregation behavior of species such as capybaras [120–122], which represent approximately 50% of total roadkill along BR-471. In the Taim Wetland, seasonal aggregation of capybaras probably begins when water levels are lower and is followed by a process of seasonal dispersion as water levels increase. This mechanism of seasonal dispersal of capybaras as a response to flooded areas is also observed in studies in the Brazilian Pantanal and the Venezuelan Llanos [121–124]. Thus, the increased movement of capybaras during periods when water levels are rising exposes this species to a greater roadkill risk, especially since the highway at a higher elevation can be used as a resting place.

Coypus are mostly nocturnal, elevating their exposure risk to roadkill [125]; however, the level of diurnal activity can also be significant during the cold season when supplemental food is required [126]. Furthermore, during winter, coypus tend to assemble in available meadows for foraging, increasing the risk of collisions, and the presence of vegetation such as hedgerows and shrubs near road edges further contributes to casualties involving coypus [127]. Conditions like these are present in Taim Wetland, where it is very common to find coypu burrows alongside the BR-471 roadside and ditches (Figure 9a), exposing this species to roadkill, resembling the circumstances reported for the European rabbits [128].

Reptile roadkill incidents were more pronounced during spring and summer, while roadkill incidents involving the birds class did not exhibit a clear seasonal pattern. The increased likelihood of reptile roadkill during summertime is a phenomenon reported in different studies [129–132]. Reptiles such as snakes and turtles are ectothermic and rely on external sources of heat to regulate their body temperature, leading them to use roads as basking surfaces or for traversing from one side of the road to the other in search of food or shelter [133], which heightens the risk of being run over.

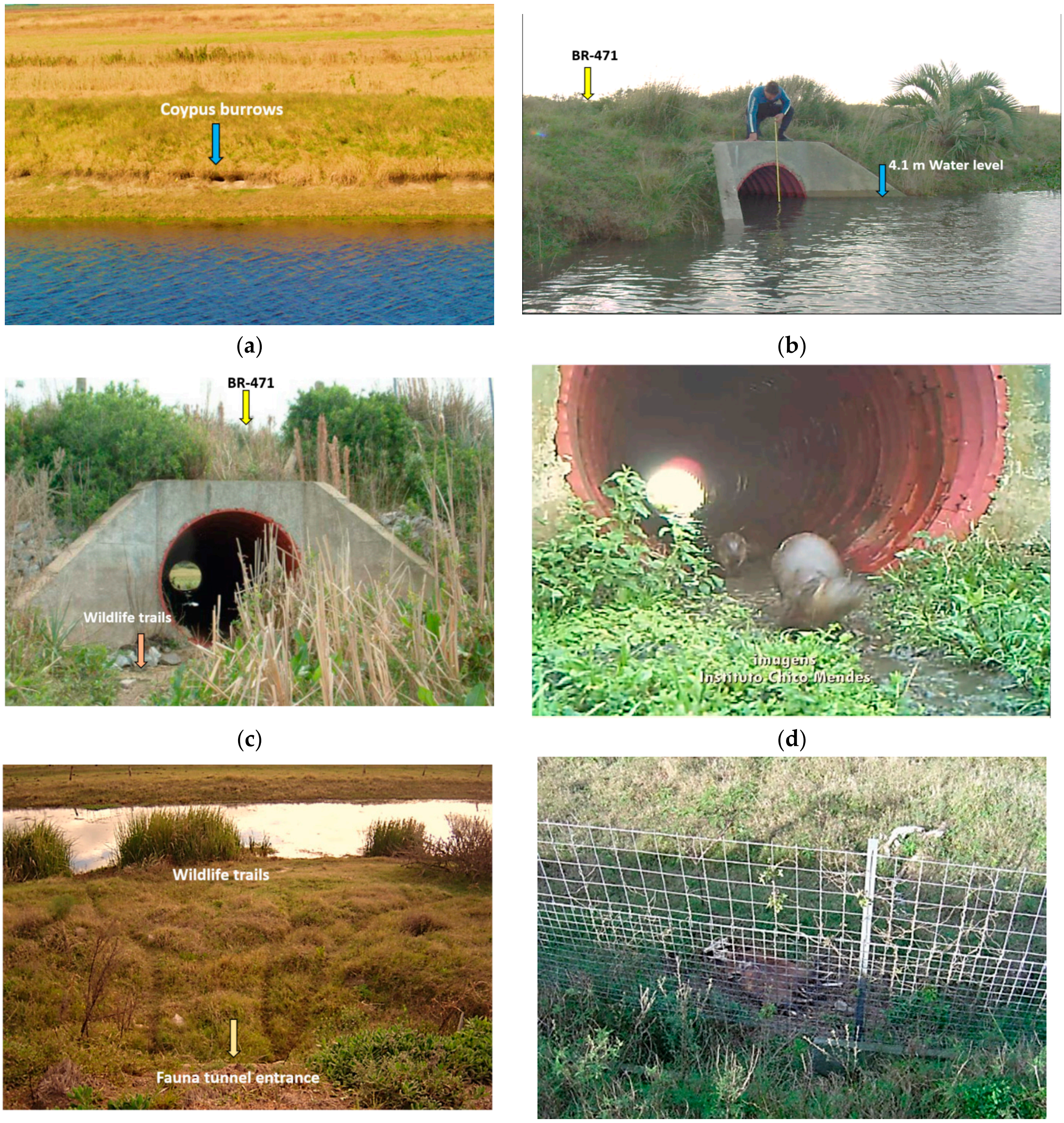
Rainfall did not prove to be a determining climatic variable for wildlife roadkill statistics, possibly due to the relatively uniform distribution of rainfall volumes throughout the months of the year, in contrast to the seasonal patterns observed for roadkill incidents. In fact, this relationship has been a subject of interest in several scientific studies with divergent conclusions. Some authors suggest that this relationship is mediated by complex interactions between rainfall itself and the vegetation cover, road conditions, and depends on the characteristics of the rainfall events, which can motivate [75,113,134–136] or inhibit animals' movement and their exposure to roadkill [117], depending on the species behavior [137,138]. Thus, other climatic information as temperature and humidity can be used in further analysis with more promising responses.

However, the analysis of the Taim Wetland water level regime allowed us to understand the seasonal behavior of roadkill and establish of a critical water level threshold for roadkill. There was clear evidence that rising water levels acts as a trigger, increasing the activity of animals, particularly herbivorous mammals such as capybaras and coypus. This, in turn, results in an increase in the incidence of wildlife roadkill along BR-471, even after the implementation of the WPS. This implies periods of amplified risk for drivers too, with potential damage to human life, especially collisions with large animals such as capybaras. In fact, the results suggest that the WPS has contributed to a reduction in total roadkill incidents, especially when the water level remains below 3.3 m. A detailed analysis of the WPS project reveals that above this water level, the fauna tunnels become partially to fully submerged (Figure 9b), potentially leading to a decrease in the WPS efficiency. During low-water-level periods, the fauna tunnels are clearly visible (Figure 9c) and easily accessible to the fauna, providing a safe crossing between the Taim Wetland and the Mirim Lake floodplain. The presence of well-defined trails (Figure 9e) detected during field observations confirm that the fauna tunnels are heavily used by coypus, capybaras, and other animals, facilitating their road crossing (Figure 9d).

Landscape characteristics at the Taim Wetland West region, along BR-471, are excellent habitats, favoring the presence of various mammals such as capybaras and coypus, as well as reptiles like yacares, turtles, and lizards, among others. Capybaras, for instance, are highly dependent on habitat condition, typically living in social groups near water bodies and land–water interfaces [122,139]. Water is essential for this species, serving as a medium during mating, as a shelter habitat, as a means of escape, and, notably, as a means to regulate body temperature [140]. Similarly, coypus inhabit dense vegetation or burrows located along vegetated banks near waterways, usually just above the waterline [141]. Consequently, when water level fluctuations alter the characteristics of their habitats [142], it results in changes in the spatial and temporal distribution of these herbivores [143,144], increasing the probability of wildlife roadkill incidents.

Figure 5 shows a high number of mammal roadkill at the beginning of the monitoring period, with incidents primarily linked to coypus (approximately 80% of the roadkill incidents in the first two months of monitoring). In August 1995, for example, a total of 243 roadkill incidents were recorded, with approximately 47% of these incidents involving coypus. At this time, there was no WPS in place, and the average water level was approximately 2.7 m. When examining the period prior to this month, it was observed that the Taim Wetland was experiencing an extremely severe drought, which began around 1988.

Therefore, it is believed that the conditions resulting from the extended drought period may have led coypus to construct their burrows close to watercourses under low-water-level conditions. When water levels rose, these burrows were flooded, leading to a significant dispersal of the species (this phenomenon was also observed in 2005) and the high rates of roadkill incidents recorded for this species.



(**a**) (**b**) (**c**) (**d**) (**e**) (**f**)

Figure 9. Coypu burrows (**a**), fauna tunnel partially submersed in Jun 2002 (**b**), low water level in May 2005 (**c**), coypus crossing a fauna tunnel during low water level (**d**), wildlife trails at the fauna tunnel entrance (**e**), and capybara carcass near the fence (**f**). Source: pictures (**a**,**c**,**e**,**f**) belong to the first author's personal collection; (**b**) was provided by [85]; (**d**) was taken from a video recorded by ICMBIO staff.

The period following the implementation of the WPS shows that the pattern of roadkill incidents follows the trend of the hydrological series, especially during the rising water levels in winter, as previously discussed. This pattern is likely associated with the seasonal behavior of the fauna, coupled with the ineffectiveness of the fauna tunnels due to their submersion. In a flood situation, it is important to consider that, with the presence of the fences, animals (particularly larger ones) unable to pass through it may end up perishing within the area, and these numbers were not accounted for in this analysis. For instance, in November 2002, despite a low number of roadkill incidents reported (water level ~4.0 m), during a field visit, several dead capybaras were photographed near the fences (Figure 9f), indicating an apparent attempt to escape.

A report [145] has already mentioned possible issues related to the submersion of fauna tunnels because the positioning of the fences in the WPS was considered inadequate, drastically reducing the dry area between the fences and the road embankment, thus diminishing escape options during flood periods and promoting agonistic behavior among individuals of some species. Additionally, the mesh spacing of the fences caused the entanglement of certain species during their attempts to escape. Therefore, it appears that the wildlife protection system, intended to facilitate communication between both sides of the highway, seems to be effective only during low water level situations. There is also a deficiency in the WPS caused by the absence of a protective fence along a section of the road, which allows animals to freely cross BR-471, consequently increasing the number of roadkill incidents.

A family of GAMLSS models was used to investigate whether it is possible to model the monthly occurrence of roadkill incidents over time and forecast the forthcoming periods with higher probability of incidents. The results indicate that, with the exception of birds, water level was the most significant variable shaping roadkill patterns, in alignment with the time series frequency analysis. The models developed demonstrated a reasonably accurate reproduction of total roadkill incidents and those related to the mammals class, including the individual models for capybaras, coypus, and other mammals. Models for birds and reptiles displayed poor predictive performance, likely due to the absence of a distinct mortality pattern for these classes, or possible due to a limited number of recorded roadkill incidents involving species within these categories.

Further research endeavors could seek to enhance the developed models by incorporating additional meteorological data such as temperature and humidity, roadkill hot-spots, fire occurrences, among others. Additionally, lagged-time series data, such as the rainfall and evapotranspiration, could further improve model accuracy.

This study complements previous research aimed at comprehending the disturbing rates of wildlife roadkill along BR 471 within the Taim Wetland region. To date, it represents the first research effort in the Taim Wetland area that put the long-term water level data in a perspective for assessing wildlife roadkill, highlighting the role of the hydrological regime as a key component that triggers fauna movements, increasing roadkill incidents. In this context, it is essential to emphasize the robustness of the 2D hydrological/hydrodynamic model employed. Its noteworthy performance indicators ensured a consistent extension of the water level time series, enabling the determination of a comprehensive long-term hydrological series that would otherwise be unavailable for integrated data analysis.

5. Conclusions

Our findings underscore the significant influence of the Taim Wetland's hydrological dynamics on roadkill incidents, implying the existence of a water level threshold beyond which fauna movements increase and roadkill risk intensifies. A comprehensive understanding of environmental seasonality, species vulnerability, and water level in the Taim Wetland is essential to enhance the efficacy of wildlife roadkill mitigation measures.

In this context, integrating hydrological modeling results with statistical models emerges as a valuable approach for predicting wildlife patterns and anticipating peaks in mortality. We believe that such tools hold considerable promise as assets in formulating multifaceted strategies to reduce the prevalence of wildlife roadkill, optimizing resources. These approaches could encompass public awareness campaigns, regulatory enforcement, enhanced road signage, and speed control, all aligned with forecasts generated from these integrated models.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. However, the original database can be obtained at ICMBIO-IBAMA, ESEC-Taim.

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

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