Anuran road-kills neighboring a peri-urban reserve in the Atlantic Forest, Brazil

Igor Pfeifer Coelho, Fernanda Zimmermann Teixeira, Patrick Colombo, Artur Vicente Pfeifer Coelho, Andreas Kindel

Abstract
Mortality from road-kills may figure among the important causes of decline in amphibian populations and species extinctions worldwide. Evaluation of the magnitude, composition, and temporal and spatial distributions of amphibian road-kills is a key step for mitigation planning, especially in peri-urban reserves. Once a month for 16 months, we surveyed, on foot, a 4.4 km section of state road ERS-389 bordering the Itapeva reserve in the southern Atlantic Forest. We recorded 1433 anuran road-kills and estimated a mortality rate of 9002 road-kills/km/year. The species most often recorded were the largest bordering the Itapeva reserve in the southern Atlantic Forest. We recorded 1433 anuran road-kills and estimated a mortality rate of 9002 road-kills/km/year. The species most often recorded were the largest

1. Introduction
In recent decades, population declines and species extinctions in amphibian assemblages have been documented in many regions of the world, and amphibians have been recognized as one of the most threatened animal groups in the ongoing biodiversity crisis (Stuart et al., 2004). Nowadays, 41% of amphibian species that have been adequately evaluated are considered to be threatened, a much higher proportion than estimates for birds and mammals (IUCN, 2011). Singly or in combination, climate change, habitat loss, habitat fragmentation and splitting, invasive species introduction, increases in UV-B radiation and infectious diseases (especially chytridiomycosis), chemical pollution, and overexploitation are the main factors threatening amphibians (Semlitsch, 2003; Becker et al., 2007; Pounds et al., 2006; Young et al., 2001). Wildlife-vehicle collisions, one of the main direct causes of animal mortality worldwide (Forman and Alexander, 1998), have also been associated with declines of amphibian populations (Fahrig et al., 1995) and may represent the greatest threat for some species (Elzanowski et al., 2009; Hels and Buchwald, 2001).

The majority of studies concerning amphibian road-kills have been conducted in temperate zones and developed countries, and knowledge about estimated mortality, spatio-temporal patterns, and associated factors is limited to these regions (Puky, 2006). The high mortality rates observed (e.g. Ehmann and Cogger, 1985, estimated that 4.45 million anurans are killed on roads each year in Australia) may also be occurring in other regions. South America is the continent with the highest number and density of amphibian species (Duellman, 1999), and harbors 35.9% of threatened amphibian species (IUCN, 2011). However, local evaluations of amphibian road mortality are still rare in South America, although this source of mortality may have an important impact on populations.
a road through the Argentinean Pampas has a significant impact on the local population of *Melanophryniscus* sp., with an estimated annual road-kill mortality of up to 5.9% of the population.

Effectively managed systems of protected areas are thought to be crucial for protecting amphibians from human activities (Young et al., 2001). However, the ongoing extension of human occupation over landscapes leads to expansion of infrastructure such as roads near protected areas, which may compromise the functionality and effectiveness of these areas for conservation. Since roads near urban areas carry heavy and continuous vehicle traffic, the impact of neighboring roads on small and peri-urban reserves may be extremely severe (Ramp et al., 2006). It is critical to evaluate spatial and temporal patterns of road-kill in these situations, to provide information to use in planning mitigation measures, especially for threatened groups such as amphibians.

We describe the species composition and estimate the magnitude of anuran road-kill on a road bordering a peri-urban reserve in the Atlantic Forest of southern Brazil. We analyze the spatial and temporal distributions of anuran road-kills in general, and for the individual species that were most often recorded (*Leptodactylus latrans*, *Rhinella icterica*, *Leptodactylus gracilis* and *Hypsiglena sp.*), identifying which factors are responsible for the higher rates of accidents at certain times and locations. We discuss the impact of mortality in a regional context, and describe mitigation measures that could be implemented locally, based on our findings.

2. Material and methods

2.1. Study area

We conducted this study near the Itapeva State Park (PEVA), in the southern part of the Atlantic Forest Biosphere Reserve, Brazil (Fig. 1). The local climate is subtropical humid, with an annual mean temperature of 19 °C and annual mean precipitation of 1400 mm. The reserve covers an area of 1000 ha, encompassing the last preserved coastal ecosystems in the region, including mobile dunes, “restinga” forest (medium sized trees and shrubs adapted to sandy soils), marshy forest, marshes, peatlands, and also human-made marshy and dry grasslands and transitional formations (Lindeman et al., 1975; Kindel, 2002). This high ecosystem diversity in a small area has led to a relatively high anuran species richness (28 spp.) for this latitude (Colombo et al., 2008).

State road ERS-389 bordering the PEVA is 11 m wide, with one lane in each direction and no shoulder. Buses and trucks are prohibited, and the speed limit is 80 km/h. Traffic volume is high in summer and low in winter, with an annual mean of 2132 vehicles per day, maximum of 4431 in January, and minimum of 1252 in July (2002 data; DAER RS www.daer.rs.gov.br).

2.2. Road-kill data

We evaluated anuran road-kills monthly, between July 2002 and October 2003. In the morning, two observers on foot surveyed (one in each road lane) a 4410 m section of ERS-389, neighboring the PEVA. Anuran carcasses found were identified, their locations determined with a GPS receiver, and removed from the road.

2.3. Variables associated with road-kill

2.3.1. Temporal variables

We tested the relationship between temporal variations of anuran road-kills and: TEMPERATURE, mean of daily mean temperature; RAINFALL, total precipitation; PHOTOPERIOD, mean number of hours of sunlight per day; VEHICLE TRAFFIC, total number of vehicles; and VEHICLE SPEED, percentages of vehicles above 40, 60 and 80 km/h.

Each variable was calculated for the entire periods of 20, 10, 5 and 2 days before the day of the survey in each month, in order to evaluate the scale dependence of these variables. We obtained data for temperature and precipitation from the meteorological station in Torres municipality (Meteorology National Institute, INMET www. inmet.gov.br), 3.5 km distant from the study area. Daily sunlight hours were obtained from the Brazilian National Observatory (http:// euler.on.br/ephemeris/index.php). To calculate traffic volume and vehicle speed, we used data from a speed monitor located on the road section surveyed (DAER RS - www.daer.rs.gov.br).

2.3.2. Spatial variables

We investigated the association between road-kills and the areas of the different classes of land cover: WATER, rivers and lakes; MARSH; URBAN; houses and other buildings; AGRICULTURE; MARSHY FOREST; GRASSLAND; PASTURE; SHRUBS; PAVED ROAD; UNPAVED ROAD; MANAGED WOODS, woods with alien and/or native fruit trees without an understory; EXPOSED SOIL; and SILVICULTURE, Eucalyptus sp. Other spatial variables evaluated were: ROAD DITCHES, length of ditches up to 20 m from the road; ARTIFICIAL LIGHT, light intensity considering the number of street lights and their distance from the road; VEHICLE SPEED, mean vehicle speed on the road segment; WATERBODY DISTANCE, distance to the nearest perennial waterbody.

We divided the road into 63 segments of 70 m each, and calculated the spatial variables for each of the 64 points that delimited these segments. We used ArcMap 9.2 software (ESRI, Redlands, California) and a Quick Bird image (pixel 0.6 × 0.6 m) classified by visual interpretation (Dobrovolski, 2006) to obtain the classes of land cover in buffers of 50, 100 and 200 m radius centered on each of the 64 points. The variables ROAD DITCHES, ARTIFICIAL LIGHT, and VEHICLE SPEED were measured in the field with a GPS receiver and calculated for each point, using a buffer of 50 m radius.
on GPS TrackMaker software (www.trackmaker.com). Mean vehicle speed on each road segment was estimated using the mean speed of vehicles \((n = 19)\) that we followed by car, recording the speed during the route with a GPS.

2.4. Statistical analyses

2.4.1. Temporal distribution of road-kills

We evaluated the temporal distribution of road-kills for all anurans and for species with high numbers of records, through circular analysis (Zar, 1999). Using ORIANA 2.02 software (Kovach, 2004), we converted the months \((n = 16)\) into angles (22.5 grade intervals) and used the number of road-kills in each month as the frequency of each angle, obtaining a mean angle that represents the average period with the highest mortality during the time interval evaluated. The Rayleigh Uniformity test was performed to evaluate the significance of the average period in relation to a uniform distribution of records (Kovach, 2004; Zar, 1999). We also estimated the intensity of road-kill concentration by the length \((r)\) of the average period, which varies from 0 (uniform dispersion) to 1 (concentration of records in the same direction).

2.4.2. Spatial distribution of road-kills

The Ripley’s K statistic is used to evaluate the dispersion of events on different spatial scales (Clevenger et al., 2003; Levine, 2000; Ripley, 1981). We used a modified Ripley’s K (Coelho et al., 2008) in SIRIEMA v1.0 software (www.ufg.br/biociencias/siriema) to determine the scales on which road-kills were significantly aggregated in space. To define the different scales evaluated, we used an initial radius of 50 m and increments of 50 m for each step. To evaluate the significance of possible aggregations, we subtracted the observed \(K\) values from the mean obtained in 1000 simulations of random road-kill distributions for each scale. Values above the confidence limits (99%) obtained from the simulations indicate scales with significant aggregations (Levine, 2000).

We located the road stretches with high mortality using 2D HotSpot Identification analysis in SIRIEMA v1.0 software. In this analysis, the road is divided into segments of the same length (we used 63 segments of 70 m each, delimitated by 64 points). A circle with radius \(r\) (we used a 50 m radius) is centered on the first of these 64 points, and all road-kill events inside the circle area are summed. This sum is multiplied by a correction factor that considers the length of the road inside the circle in this position. Then, the circle is centered on the next point and the sum is again computed and multiplied by the correction factor. This procedure is repeated for all 64 points delimiting the road segments, resulting in an aggregation intensity value for each road segment:

\[
H_i(r) = \frac{2r}{C_i(r)} \sum_{j=1}^{n} f_{ij}
\]

where: \(H_i(r)\) = aggregation value for point \(i\) considering scale \(r\); \(n\) = number of road-kill events; \(r\) = defined radius; \(i\) = point on road; \(f_{ij}\) = road-kill event; \(C_i(r)\) = road length inside the circle, with \(r\) radius centered on point \(i\); \(f_{ij}\) = index equal to 0 if \(j\) is outside the circle with \(r\) radius centered on \(i\), or equal to 1 if \(j\) is inside this area. To evaluate the significance of the aggregation intensity, for each point the following function is used:

\[
I(r) = H_i(r) - Hs(r),
\]

where: \(Hs(r)\) = mean of \(H\) values in 1000 simulations of random distribution of the events. Values for aggregation intensity above the upper confidence limit (90%) indicate significant road-kill hotspots.

2.4.3. Association between road-kills and explanatory variables

We transformed temporal and spatial explanatory variables into \(X' = \log(X + 1)\). We included in the multiple regression analyses only those variables that showed a significant linear correlation (Pearson’s correlation coefficient, \(P < 0.05\)) with road-kills. We used two multiple regression approaches (path analysis and model selection using AIC) to evaluate the relationships between explanatory variables and all anuran road-kills, and separately, between explanatory variables and road-kills of the species recorded most often (\(L.\ latrans, R.\ icterica, L.\ gracilis\) and \(H.\ faber\)).

We carried out path analyses to identify the relative importance of the direct and indirect influences of each temporal variable on the temporal distribution of mortality, since some explanatory variables have a known influence on other explanatory variables (PHOTOPERIOD and RAINFALL on TEMPERATURE). First, we tested in which period (20, 10, 5 or 2 days) each variable showed the highest significant association with road-kills, using the highest Pearson’s correlation coefficient obtained. We calculated path coefficients, their significance, and the model fit using the maximum-likelihood estimate in AMOS 5.0.1 software (Arbuckle, 2007). Model fit was tested by Chi-square test. The normality of each variable was evaluated observing skewness and kurtosis values, and multivariate normality was tested using kurtosis (Mardia’s coefficient values below 1.96 indicate that there is no significant kurtosis; Mardia, 1970). For \(H.\ faber\), we also show the standardized regression coefficients in a path diagram, although TEMPERATURE did not show a significant correlation with road-kills, and was not used in the model.

Since there are no known relationships among the spatial explanatory variables (and we had no hypotheses about these relationships to test), we performed a model selection using the second-order Akaike information criterion (AICc) to evaluate which variables were more related to road-kills in space. Considering 64 sample units (the 64 points that limit the road segments of 70 m each), we first evaluated on which scales (buffers with a radius of 50, 100 and 200 m) the land cover classes showed the highest Pearson’s correlation coefficient with the intensity of road-kill aggregation. Correlations between selected spatial variables were not superior to 0.75. All possible models for each response variable and its set of explanatory variables were ranked according to lower AICc scores, and the Akaike weight for each model \((\omega_m)\) was estimated using SAM software (Rangel et al., 2010). Models with an AICc difference lower than two when compared with the lowest one were considered as plausible models (Burnham and Anderson, 2002). The importance of each explanatory variable was calculated by the sum of the Akaike weights over the subset of models were the variable was included \((\sum\omega_m)\), and the standardized regression coefficients \((\hat{b}_i)\) were estimated using model averaging across all models (Burnham and Anderson, 2002).

2.4.4. Estimate of annual mortality magnitude

We performed two estimates of anuran annual mortality using multiple regression, considering the predictive variables identified in the path analysis as having a significant direct influence on anuran road-kills. Because carcasses decompose with time and vehicle traffic, and are removed by scavengers, they remain on the road for a very short time, less than 24 h (Teixeira, 2011), or on the order of 1 or 2 (Santos et al., 2011) or 3.2 days (Gerow et al., 2010). For this reason, we defined pseudo-samplings for each day (total of 365 days) or every three days (total of 121 days) during the period from July 2002 through June 2003. We estimated the road-kill number for each pseudo-sampling using the values from predictive variables for those days in the multiple regression equation obtained in the path analysis. The sum of all road-kills observed during the real sampling days and the road-kills estimated for
pseudo-sampling days was used to estimate the total annual mortality.

For the purpose of comparing between methods, we also estimated mortality using the equation proposed by Teixeira (2011):

\[ N = np\lambda TR \]

where \( N \) = total number of road-kills observed, \( n \) = number of surveys, \( p \) = detection probability, \( \lambda \) = mortality rate (road-kill number per time unit), and \( TR \) = characteristic time for carcass removal. This equation was developed in order to correct for bias in road-kill estimates caused by carcass removal and searcher detection. We used \( TR \) value (0.964 days) obtained for anurans by Teixeira (2011) on a road near ERS-389 (approximately 30 km to the southwest) and a detection probability of 0.9 (close to the maximum detectability value, considering that the survey was performed on foot). We calculated the daily mortality rate (\( \lambda \)) and multiplied it by 365 to generate an annual estimate.

3. Results

We recorded a total of 1433 anuran road-kills, belonging to 13 species and six families (Table 1). The majority of records (54.7% of the total) were not identified to species, and 35% could not be identified even to family level, due to carcass disintegration. The family Leptodactylidae had the highest number of records (35.7%). The species most often recorded were \( L. \) latrans (18.9%), \( R. \) icterica (6.8%), \( L. \) gracilis (6.7%) and \( H. \) faber (6.6%).

3.1. Temporal distribution of road-kills and related factors

Anuran road-kills were significantly concentrated (Rayleigh’s Uniformity Test, \( Z = 601.9, P < 0.001 \)) in the summer months, with a mean concentration period estimated in January (\( r = 0.64 \); Fig. 2a). Individual species mortality was also concentrated in some periods (Fig. 2b, c, d, e). For \( L. \) latrans \( (Z = 203.4, P < 0.001; r = 0.86), L. \) gracilis \( (Z = 38.5, P < 0.001; r = 0.63) \) and \( H. \) faber \( (Z = 71.9, P < 0.001; r = 0.87) \) there were also more records during summer, and the mean periods with the highest road-kill occurrence estimated were in February (Fig. 2b), between December and January (Fig. 2d) and in December (Fig. 2e), respectively. \( R. \) icterica mortality was more evenly distributed through the year \( (Z = 35.8, P < 0.001; r = 0.60) \), with the highest mortality in October 2002 (Fig. 2c).

Path diagrams that describe the relationships among temporal variables and road-kills are presented in Fig. 3. Models for Anura \((\chi^2 = 0.11; DF = 1; P = 0.73), L. \) gracilis \((\chi^2 = 2.25; DF = 2; P = 0.32) \) and \( H. \) faber \((\chi^2 = 0.99; DF = 2; P = 0.60) \) showed a good fit to the data. The model for \( L. \) latrans showed a significant Chi-square, which indicates a lack of satisfactory model fit \((\chi^2 = 7.23; DF = 1; P = 0.007) \). However, we used this model for interpretation because the variables were highly correlated, making a poor fit and leading to a Type II error of inference in the Chi-square test. For the \( R. \) icterica model, there was no value of positive degrees of freedom to calculate the model fit.

Temperature showed the strongest effect on anuran road-kills (standardized path coefficient = 0.63), followed by precipitation (0.45). Vehicle traffic did not show a significant direct effect on anuran road-kills in this evaluation, considering the other variables. Photoperiod, although it did not show a significant direct effect on mortality, showed an indirect effect through the strong influence on temperature (0.88). Although vehicle traffic did not show a significant influence on anurans as a whole, evaluations of individual species showed that it influenced road-kills of \( L. \) gracilis and \( L. \) latrans (standardized path coefficient = 0.56 and 0.50, respectively). Temperature directly influenced only \( L. \) latrans road-kills, but photoperiod (which showed an indirect relationship with temperature in all cases, except for \( R. \) icterica since temperature did not enter into the model) directly influenced \( R. \) icterica (0.38) and \( H. \) faber (0.57). Precipitation, although it showed a significant direct relationship with anurans in general, for the individual species separately, significantly influenced only the mortality of \( R. \) icterica (0.51).

3.2. Spatial distribution of road-kills and related factors

Road-kill spatial distribution for anurans in general and for the species separately on state road ERS-389 was non-random for almost all scales evaluated (Fig. S1 in Supplementary material), indicating that the records were concentrated in some locations along the road section. The location of road-kill hotspots for anurans and for the individual species differed along the road (Fig. 4), indicating that different factors may be more important for mortality occurrence in each case.

The model selection provided 18 models for anuran road-kills \((R^2 = 0.53), five for \( L. \) latrans \((R^2 = 0.64), three for \( R. \) icterica \((R^2 = 0.47), 13 for \( L. \) gracilis \((R^2 = 0.56), and three for \( H. \) faber \((R^2 = 0.23) \), which could be considered as similarly plausible according to their AICc (Table S1). Table 2 shows the selected variables for predicting spatial distributions of road-kills, its relative importance and the standardized regression coefficients. Anuran mortality was mainly associated with WATERBODY DISTANCE \((\gamma_{0} = 0.966, \beta = 0.48)\) and WATER \((\gamma_{0} = 0.921, \beta = 0.28)\); ROAD DITCHES \((\gamma_{0} = 0.999, \beta = 0.45)\), PASTURE \((\gamma_{0} = 0.969, \beta = 0.37)\), and UPAVED ROAD \((\gamma_{0} = 0.991, \beta = 0.40)\) were the most important variables related to \( L. \) latrans road-kills. ARTIFICIAL LIGHT \((\gamma_{0} = 0.987, \beta = 0.44)\), WATERBODY DISTANCE \((\gamma_{0} = 0.738, \beta = 0.28)\), MANAGED WOODS \((\gamma_{0} = 0.703, \beta = 0.23)\), SILVICULTURE \((\gamma_{0} = 0.674, \beta = 0.25)\), and GRASSLAND \((\gamma_{0} = 0.625, \beta = 0.20)\) were identified as the main predictors for \( R. \) icterica mortality. \( L. \) gracilis road-kills were mostly related to SHRUBS \((\gamma_{0} = 0.999, \beta = 0.40)\), ROAD DITCHES \((\gamma_{0} = 0.966, \beta = 0.33)\), and

### Table 1

<table>
<thead>
<tr>
<th>Species of road-killed anurans on state road ERS-389 bordering the Itapeva Reserve, southern Brazil.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taxon</strong></td>
</tr>
<tr>
<td>Buitonidae</td>
</tr>
<tr>
<td>Rhinella icterica</td>
</tr>
<tr>
<td>Buitonidae NI</td>
</tr>
<tr>
<td>Microhylidae</td>
</tr>
<tr>
<td>Elachistocleis bicolor</td>
</tr>
<tr>
<td>Hylidae</td>
</tr>
<tr>
<td>Hypsiboas faber</td>
</tr>
<tr>
<td>Hypsiboas guentheri</td>
</tr>
<tr>
<td>Scinax fusovarius</td>
</tr>
<tr>
<td>Scinax granulatus</td>
</tr>
<tr>
<td>Scinax squamipilosus</td>
</tr>
<tr>
<td>Sphaeromolochus aff. surdus</td>
</tr>
<tr>
<td>Hylidae NI</td>
</tr>
<tr>
<td>Leptodactylidae</td>
</tr>
<tr>
<td>Leptodactylus gracilis</td>
</tr>
<tr>
<td>Leptodactylus latrans</td>
</tr>
<tr>
<td>Leptodactylidae NI</td>
</tr>
<tr>
<td>Cyclorhaphidae</td>
</tr>
<tr>
<td>Odontophrynus matsuma</td>
</tr>
<tr>
<td>Leipuridae</td>
</tr>
<tr>
<td>Physalaemus gracilis</td>
</tr>
<tr>
<td>Physalaemus lifni</td>
</tr>
<tr>
<td>Leipuridae NI</td>
</tr>
<tr>
<td>Anura NI</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>Species richness</strong></td>
</tr>
</tbody>
</table>

NI — not identified.
WATER ($\sum \omega_i = 0.962$, $\beta = 0.31$). The models for $H. faber$, which explained a small amount of variation in road-kills, showed AGRICULTURE ($\sum \omega_i = 0.956$, $\beta = 0.34$) and MANAGED WOODS ($\sum \omega_i = 0.765$, $\beta = 0.27$) as the main explanatory variables.

3.3. Anuran road-kill estimates

The predictor variables TEMPERATURE and RAINFALL were used in the equation $X = -6.69 + 1.17 \times \text{Rainfall} + 4.67 \times \text{Temperature}$
\[ R^2 = 0.82, F_{2,13} = 36.16, P < 0.001 \] to estimate anuran annual mortality on roads. We estimated that the annual road-kill was on the order of 36,011 (9002 road-kills/km/year) when considering one day of pseudo-sampling interval, and 11,233 individuals (2547 road-kills/km/year) when considering three days. Based on the equation proposed by Teixeira (2011), the estimated mortality was 37,683 anurans per year (9420 road-kills/km/year).

**4. Discussion**

**4.1. Anuran road-kill magnitude and composition**

Amphibians are the vertebrate group with the highest mortality from vehicle collisions in many regions (Attademo et al., 2011; Carvalho and Mira, 2010; Gerow et al., 2010; Glista et al., 2008; Gryz and Krauze, 2008; Turci and Bernard, 2009), and may be the group most impacted by this factor worldwide. Observed annual road-kill rates per kilometer for anurans may be on the order of 3.4 (gravel and dirt roads, Coleman et al., 2008), 245 (this study), or 246 (Attademo et al., 2011), reaching 21,940 road-kills/km/year (Goldingay and Taylor, 2006), although comparisons among these studies are biased due to differences in sampling intervals. Observed road-kill values, however, are certainly underestimates of real mortality. Mortality estimates based on observed road-kills, especially considering observers' detection capabilities and carcass persistence (Gerow et al., 2010; Santos et al., 2011; Teixeira, 2011), are needed to evaluate the magnitude of this impact and to compare the impacts among different roads and regions. The method that we used to estimate road-kill, using predictive variables, yielded a similar result to that obtained using observers' detection and carcass persistence values (9002 and 9420 road-kills/km/year, respectively), indicating that it can be used in the absence of experiments on detection and removal rates, when predictive variables that explain a substantial amount of temporal variability in mortality are identified (in this case, 82%). The only study that also estimated anuran mortality rate is Gerow et al. (2010), who...
estimated 159.3 road-kills/km/year in the desert of Saguaro National Park, Arizona.

A large part of the anuran assemblage in the PEVA region is subject to road-kill impact; 46% of the known species in the reserve and 56% of the species that occur in habitats near the road have been recorded as road-kill (Colombo et al., 2008). The anuran species most vulnerable to road-kills are those with terrestrial habits, high vagility, long migration distances during the breeding season, low rates of movement, philopatry to reproductive sites, and a daily movement pattern that coincides with peaks of traffic flow (Carr and Fahrig, 2001; Hels and Buchwald, 2001; Orlovski et al., 2008; Sillero, 2008). Species with high numbers of records on ERS-389 are common, and are generalists capable of exploiting a wide range of environmental conditions. Silva et al. (2007) found

Fig. 4. Road-kill intensity of aggregation (black line) and 90% confidence limits (light-gray lines) along 4.41 km of ERS-389: (a) anurans, (b) *Leptodactylus latrans*, (c) *Rhinella icterica*, (d) *Leptodactylus gracilis* and (e) *Hypsiboas faber*. Values above the upper confidence limit indicate significant hotspots of mortality.
similar results to ours, in a study in another area in the southern Atlantic Forest, where *L. latrans* was also the species with the highest number of road-kills. In other studies of anurans in Brazil, the genus *Rhinella* showed the highest road-kill frequency (reviewed by Dornas et al., 2012); it is also the genus most often correlated with road-kills, or were not selected in the plausible models subset (see Methods).

**4.2. Factors related to temporal distribution of road-kills**

Variables associated with organisms' activity (especially temperature, but also precipitation and photoperiod) were the most important factors related to the temporal distribution of anuran road-kills overall, and to road-kills of *L. latrans*, *R. ictericus*, and *H. faber*. High dermal permeability and ectothermy are the main physiological characteristics driving anuran behavior and ecology, making these organisms’ activity pattern, movements, and locomotor performance closely related to temperature and water availability (Wells, 2007). Photoperiod is also important in the regulation of anuran activity rhythms (Wells, 2007), and is recognized as a determinant factor in anuran reproductive activity in southern Brazil (Both et al., 2008). Other studies demonstrated a positive relationship of anuran road-kills to temperature and precipitation (Attademo et al., 2011), with road-kill peaks during adult migration periods in spring, and juveniles during summer and autumn (Grycz and Krauze, 2008; Orłowski, 2007; Orłowski et al., 2008).

**Leptodactylus** road-kills were strongly influenced by vehicle traffic. In the study area, *Leptodactylus* spp. were abundant throughout the year, even outside the breeding season (P. Colombo, pers. obs.), probably making variations in vehicle traffic the main determinant for the number of road-kills. Mazeroni (2004) observed that even small temporal changes in the traffic volume on a road (e.g., 5–26 vehicles/h) may increase mortality for some amphibian species (e.g., *Bufo americanus*); whereas for others (e.g., *Pseudacris crucifer*), the number of road-kills decreases with increasing traffic, probably because the animals avoid the road when traffic is heavy. Vehicle traffic may influence amphibian road-kills in different ways, increasing the number of accidents until a traffic volume threshold is reached (see the relationship between moose *Alces alces* and traffic described by Seiler, 2005) in cases when species do not avoid the road, or decreasing the species’ occurrence when anurans avoid roads with moderate to heavy traffic. The only study that directly evaluated road avoidance for an anuran species is that of Bouchard et al. (2009), who concluded that high mortality of *Rana pipiens* was due to failure to avoid roads or traffic.

**4.3. Factors related to spatial distribution of road-kills**

Spatial variations of vertebrate road-kills may be a result of the local abundance of species (Seiler, 2005), local traffic volume (Orłowski and Nowak, 2006), and/or factors that increase the

---

**Table 2**

Relative importance based on Akaike weights (\(\sum \omega_i\)) and standardized regression coefficients (\(\hat{\beta}_i\)) estimated from model averaging for predictor spatial variables of road-kill intensity of anurans as a group, and of single species. Those variables without values in each column were not included in the analysis, due to the lack of significant linear correlation with road-kills, or were not selected in the plausible models subset (see Methods).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anura (\sum \omega_i) (\hat{\beta}_i) (SE)</th>
<th><em>L. latrans</em> (\sum \omega_i) (\hat{\beta}_i) (SE)</th>
<th><em>R. ictericus</em> (\sum \omega_i) (\hat{\beta}_i) (SE)</th>
<th><em>L. crucifer</em> (\sum \omega_i) (\hat{\beta}_i) (SE)</th>
<th><em>H. faber</em> (\sum \omega_i) (\hat{\beta}_i) (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARTIFICIAL LIGHT</td>
<td>(0.999 \quad 0.45 ) (20.3)</td>
<td>(0.987 \quad 0.44 ) (8.4)</td>
<td>(0.966 \quad 0.33 ) (14.7)</td>
<td>(0.966 \quad 0.33 ) (14.7)</td>
<td>(0.966 \quad 0.33 ) (14.7)</td>
</tr>
<tr>
<td>ROAD DITCHES</td>
<td>(0.029 \quad 0.10 ) (33.8)</td>
<td>(0.326 \quad -0.15 ) (7.2)</td>
<td>(0.563 \quad -0.25 ) (8.2)</td>
<td>(0.373 \quad 0.18 ) (0.5)</td>
<td>(0.373 \quad 0.18 ) (0.5)</td>
</tr>
<tr>
<td>WATER DRAINAGE</td>
<td>(0.292 \quad -0.48 ) (8.7)</td>
<td>(0.536 \quad 0.20 ) (0.7)</td>
<td>(0.738 \quad -0.28 ) (0.83)</td>
<td>(0.373 \quad 0.18 ) (0.5)</td>
<td>(0.373 \quad 0.18 ) (0.5)</td>
</tr>
<tr>
<td>WATER (100 m)</td>
<td>(0.259 \quad -0.06 ) (0.47)</td>
<td>(0.096 \quad 0.37 ) (6)</td>
<td>(0.716 \quad 0.26 ) (2.6)</td>
<td>(0.266 \quad 0.06 ) (0.34)</td>
<td>(0.266 \quad 0.06 ) (0.34)</td>
</tr>
<tr>
<td>GRASSLAND (50 m)</td>
<td>(0.387 \quad 0.15 ) (14)</td>
<td>(0.162 \quad -0.20 ) (0.8)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
</tr>
<tr>
<td>PAVED ROAD (100 m)</td>
<td>(0.039 \quad -0.10 ) (42.2)</td>
<td>(0.657 \quad -0.19 ) (1.5)</td>
<td>(0.096 \quad 0.37 ) (1.9)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
</tr>
<tr>
<td>MANAGED WOODS (50 m)</td>
<td>(0.091 \quad -0.40 ) (3.1)</td>
<td>(0.229 \quad -0.03 ) (0.11)</td>
<td>(0.703 \quad 0.23 ) (0.69)</td>
<td>(0.999 \quad 0.40 ) (0.23)</td>
<td>(0.765 \quad 0.27 ) (0.83)</td>
</tr>
<tr>
<td>MANAGED WOODS (200 m)</td>
<td>(0.039 \quad -0.10 ) (42.2)</td>
<td>(0.657 \quad -0.19 ) (1.5)</td>
<td>(0.096 \quad 0.37 ) (1.9)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
<td>(0.212 \quad 0.001 ) (3)</td>
</tr>
<tr>
<td>SHRUBS (50 m)</td>
<td>(0.435 \quad -0.14 ) (0.45)</td>
<td>(0.674 \quad -0.25 ) (0.6)</td>
<td>(0.671 \quad -0.20 ) (0.83)</td>
<td>(0.586 \quad 0.21 ) (1.2)</td>
<td>(0.586 \quad 0.21 ) (1.2)</td>
</tr>
<tr>
<td>MARSHY FOREST (200 m)</td>
<td>(0.262 \quad -0.07 ) (0.6)</td>
<td>(0.238 \quad 0.01 ) (0.23)</td>
<td>(0.568 \quad -0.22 ) (0.39)</td>
<td>(0.956 \quad 0.34 ) (0.82)</td>
<td>(0.956 \quad 0.34 ) (0.82)</td>
</tr>
<tr>
<td>EXPOSED SOIL (200 m)</td>
<td>(0.479 \quad -0.16 ) (3)</td>
<td>(0.625 \quad -0.24 ) (1)</td>
<td>(0.267 \quad 0.06 ) (0.31)</td>
<td>(0.325 \quad -0.10 ) (0.2)</td>
<td>(0.325 \quad -0.10 ) (0.2)</td>
</tr>
</tbody>
</table>
probability of collision, such as vehicle speed (Hobday and Minstrell, 2008; Seiler, 2005). We did not evaluate the spatial influence of vehicle traffic because we considered that traffic volume did not vary along the road, since it is a short section connected to a few dirt roads used by local residents. However, we detected an association of anuran and single-species road-kills with types of land cover, waterbody distance, road ditches, and artificial light, indicating the importance of local, fine-scale anuran abundance for the occurrence of mortality hotspots. Local anuran abundance is related to landscape composition and structure (Gray et al., 2004; Knutson et al., 1999), among other factors. For members of the family Ranidae, forest area and wetland area are related to road-kill increases, while the presence of residences and urban area are related to decreases in road-kill numbers (Glista et al., 2008). Langen et al. (2009) showed that the presence and configuration of wetlands within 100 m from roads are associated with anuran road-kill hotspots, and are more important than traffic volume. Mortality of the common toad (Bufo bufo) is associated with local population abundance and with the area of waterbodies adjacent to roads, but not with traffic volume (Orlowski, 2007). However, Orlowski (2007) suggested that the lack of a relationship is due to a long-term cumulative effect of mortality on roads with heavy traffic, causing the local decline of populations, as in the case of Pelobates fuscus in Sweden (Nystöm et al., 2007).

The association between R. icterica road-kills and artificial light may be a result of high concentrations of individuals of this species searching for prey near street lights. Some anuran species use areas with artificial lights to forage, and common toads (B. bufo) concentrate close to street lights to capture insects (Buchanan, 2006). We found no important spatial relationship between road-kills and vehicle speed, probably due to high average speed and small local variations (74 km/h and 63–83 km/h, respectively). Although vehicle speed may not be important for anuran perception of the vehicle and escape (as it is for other animals of larger size and/or with a high capacity for movement), it may be an important factor for mortality caused by sudden changes of pressure. Even without being smashed by vehicle wheels, anurans can die by blowout, a high-pressure wave created by vehicles traveling above 30 km/h (Hummel, 2001). Therefore, one question that needs to be evaluated is the relative roles of blowout and collision in causing mortality. If the proportion of anuran road-kills due to blowout is high compared to mortality due to collision, road sections with a mean vehicle speed lower than 30 km/h could show less mortality, and speed reduction may also be an efficient mitigation measure for amphibians.

5. Conclusions and management implications

Anuran mortality was concentrated in summer, and was associated with temperature, rainfall and photoperiod. Implementation of temporary mitigation measures should be a priority during hot moments, that is, periods with high concentration of road-kills (Beaudry et al., 2010). In addition to the periods already identified when anuran road-kill rates are high along ERS-389, short periods when conditions favor anuran activity (related to temperature, precipitation and photoperiod) also must be considered and forecasted using predictive models based on meteorological data, using an adaptive road-mitigation approach. The identification of daily hot moments is needed to implement mitigation approaches such as temporary traffic bans and mobile speed monitors (if speed reduction is effective; see 4.3, “blowout” discussion).

Road-kill hotspots differ for anurans as a group and for single species, and the spatial pattern of mortality is associated with factors that can be managed to diminish this impact, such as types of land cover, distance from the nearest waterbody, roadside ditches, and artificial light. A comprehensive mitigation approach should take into account mortality hotspots for anurans as a group and also for target species in selecting where to implement measures, such as passages with guide fencing and barrier fences designed especially for amphibians (Woltz et al., 2008). Spatial models considering landscape characteristics associated with mortality hotspots may also be used to determine the location of mitigation measures in other ERS-389 sections and nearby roads. The formation of road ditches and construction of artificial water-bodies near roads must be avoided since they may represent ecological traps (Robertson and Hutto, 2006) for anuran populations. Fewer street lights along roads and/or the use of light bulbs that are less attractive to insects must also be considered in order to reduce road-kill of particular anuran species.

Acknowledgments

We thank all colleagues who assisted in data collection, especially Graciela B. Horn and Sofia Zank. Ricardo Dobrovolski provided a classified satellite image made available from the Secretaria Estadual do Meio Ambiente do Rio Grande do Sul (SEMA-RS). SEMA provided lodging in the PEVA during field work. This study was partially supported by the Instituto de Biociências of UFRGS.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jenvman.2012.07.004.

References
