Daytime driving decreases amphibian road kill

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Abstract: Road kill has gradually become a common factor that has contributed to the decline of amphibians. Traffic volume is an important issue that can be used to determine the impacts of roads. However, little information is available on the time of day in which the highest road-kill ratio occurs. Hence, we conducted 77 road surveys along a 10 kilometres road in the Wanglang National Nature Reserve (NNR) to determine the temporal and spatial distribution patterns of amphibian road mortality, which is an essential step for implementing mitigation measures. A total of 298 dead individuals (*Bufo andrewsi* and *Rana chensinensis*) were observed on the road from June 2015 to October 2017. *B. andrewsi* was more vulnerable to road mortality than *R. chensinensis*, and *B. andrewsi* had the highest number of records (85.2%). Amphibian fatalities mainly occurred during the breeding season in April. Moreover, *B. andrewsi* road kill was also concentrated in June and July. There was a significantly positive correlation between amphibian road mortality and mean night traffic volume. Road-kill hotspots were non-randomly distributed throughout the study area and were mainly concentrated in the road sections near the breeding pools. Therefore, to effectively mitigate the effects of road mortality in the Wanglang NNR, measures both

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during hot moments and at hotspots should be established. First, based on road-kill hot moments, the Wangling NNR should establish temporary traffic restraints at night during the breeding season (in April) and in June and July. Second, based on road-kill hotspots, culverts should be established in the area of breeding pools adjacent to roads, and fences should be installed to guide amphibians into the culverts.

Keywords *Bufo andrewsi*, *Rana chensinensis*, Road mortality, Mitigation measures, Wanglang NNR

1. Introduction

Roads have a fatal influence on vertebrates due to collisions with vehicles, and road-kill events have long been considered to have a more serious effect than hunting on vertebrate mortality in the United States (Forman & Alexander, 1998). In particular, collision with vehicles is a major cause of amphibian mortality (Hels & Buchwald, 2001; Puky et al., 2005; Cairo & Zalba, 2007; Glista et al., 2008). It is widely recognized that amphibians are the most threatened vertebrate group (Stuart et al., 2004), with nearly 40% of living amphibian species in danger of extinction (IUCN, 2011). Moreover, amphibians are more prone to road-kill events than other vertebrates (Gryz & Krauze, 2008; Matos et al., 2012; Alroy, 2015). Hence, the extensive amounts of road mortality that results from the pervasiveness of roads have urged road planners and ecologists to implement mitigation measures (Costa et al., 2015).

The global road system has constantly expanded in recent years, and it is expected that at least 25 million kilometres of new roads will be built in the world by 2050 (Laurance et al., 2014). Road system developments have been the primary cause of amphibian declines (Fahrig et al., 1995; Carrier & Beebee, 2003; Cosentino et al., 2014). However, the construction of road mitigation structures for animals can be expensive; thus, there is a need to identify the most effective locations to implement mitigation measures (Glista et al., 2009).

Traffic volume is an important issue in determining the impacts of roads (Gibbs & Shriver; 2005; Gu et al., 2011; Grilo et al., 2015). Previous studies suggested that road-kill rates were positively associated with traffic intensity (Fahrig et al., 1995;

Gibbs & Shriver, 2002). The migratory patterns of amphibians are one of the factors that contribute to collisions with vehicles when crossing a road surface (Hartel et al., 2009; Langen et al., 2009). Long-distance movement events always occur during the amphibian breeding season, for example, amphibians migrate from overwintering sites to aquatic breeding sites, which may result in high road mortality rates (Orlowski, 2007; Semlitsch, 2008). In addition, amphibian mortality was also found to be correlated with special behavioural traits, such as their low rates of movement and remaining immobile in response to an approaching vehicle (Carr & Fahrig, 2001). Ultimately, Amphibians are unable to avoid oncoming vehicles, which increases the number of collision events (Mazerolle, 2004; Lima et al., 2015).

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To date, researchers have published a considerable number of studies that have described the effects of roads on amphibians, but there are still some unresolved problems. First, many road-kill studies have focused on highways (Hobday & Minstrell, 2008; Hartel et al., 2009; Grilo et al., 2009), and only a few studies have been conducted on low-grade highways, such as third-class highways and fourth-class highways (Orlowski, 2007; Sillero, 2008; Matos et al., 2012). However, China has experienced noticeable and dramatic increases in road construction and operation mileage in recent years (Statistical Bulletin of Transport Industry in China, 2016). By the end of 2016, the total road mileage reached 4.696 million kilometres, which was a 2.6% increase from the previous year. The density of the road network in China reached 48.92 kilometres per 100 square kilometres, and the mileage of highways classified as fourth-class highways or above was approximately 4.227 million kilometres. Third-class and fourth-class highways account for 77.2% of the total road mileage. Second, many road-kill studies have focused on large animals (Carr & Fahrig, 2001; Biggs et al., 2004; Langen et al., 2009), and little attention has been paid to small fauna that experience high rates of road mortality. For example, amphibians, which are considered one of the most vulnerable groups to road mortality, tend to suffer from direct mortality due to vehicle collisions more than other vertebrates (Goldingay & Taylor, 2006; Shwiff et al., 2007; Coelho et al., 2012). Third,

Commented [DL4]: I would challenge this, there is extensive literature on amphibians, reptiles and small mammals the rapid development of ecotourism can provide close access to Chinese reserves (Zhou et al., 2013). Unfortunately, increases in the traffic volume have accompanied ecotourism, which has a destructive influence on amphibians. Thus, it is difficult to determine how we can balance the public needs of increased road networks while reducing amphibian road-kill. Finally, few studies have assessed the influences on amphibians during certain traffic volume periods, and these assessments can reveal the correct time for determining amphibian road kill. One study conducted on the peninsula of Djursland, researchers examined the diurnal variations in the amphibian fraction of road kill and found that amphibians reached the highest peak road mortality after sunset, which may be attributed to their activity patterns (Hels & Buchwald, 2001).

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Given those deficiencies, we conducted an amphibian road-kill survey on a low-grade road. This study mainly investigated four questions. (1) Which species are the most vulnerable to vehicle collisions? (2) Does road mortality have a significant impact on amphibian breeding populations? (3) What are the temporal and spatial road kill distribution patterns for the surveyed amphibians? (4) What factors contribute to amphibian road mortality? Based on our study, we can also provide targeted information for implementing mitigation measures through the temporal and spatial distribution patterns in amphibian mortality. In addition, it is necessary to identify hot moments and hotspots of amphibian road kill.

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2. Materials and methods

2.1 Ethics Statement

The conducted research is in compliance with laws and ethical standards of the country. All animal procedures were approved by the Animal Care and Use Committee of the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB2015003). All field work with animals was conducted according to relevant national and international guide-lines. Chengdu Institute of Biology issued permit number CIB201502 for field work.

2.2 Study area

We conducted this study in the Wanglang NNR (Figure 1), which is located in Pingwu County of Sichuan Province in southwest China (103°55′–104°10′E, 32°49′–33°02′N). This reserve was established mainly for the protection of giant panda and other rare wild animals as well as their habitat. The Wanglang NNR is one of the four earliest established nature reverses for panda in China. The area of the Wanglang NNR is 322.97 square kilometres with an elevation ranging from 2300 to 4980 metres. The local climate is semi-humid, with an annual mean temperature ranging from 2.5°C to 2.9°C, and an annual mean rainfall of 859.9 millimetres. The rainfall is mainly concentrated in May, June and July.

2.3 Survey route

The survey route was approximately 10 kilometres of a two-lane asphalt road (fourth-class highway) that was 5.5 metres wide. The road was constructed in 2010 and traverses the Wanglang NNR, from the Mu-yang Chang conservation station (104.10185°E, 32.96914°N) to the Bao-zi Gou conservation station (104.15619°E, 32.90912°N). There are six breeding pools along the survey route. We conducted amphibian road-kill monitoring from June 2015 to October 2017. The vegetation near the survey road consisted of a secondary broad-leaved forest dominated by *Abies fabri* (Mast.) Craib, *Betula albosinensis* Burk and *Hippophae rhamnoides* Linnaeus.

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2.4 Study animals

There are five amphibian species in the Wanglang NNR, including one salamander (*Batrachuperus tibetanus*), one frog (*R. chensinensis*) and three toads (*B. andrewsi*, *Oreolalax chuanbeiensis* and *Scutiger boulengeri*); however, we found only two species, *B. andrewsi* and *R. chensinensis*, that were vulnerable to road mortality. The other species are alpine species that are often found in mountain streams and were never encountered as road mortality along the survey road. The road runs through the habitats of *B. andrewsi* and *R. chensinensis*, which may pose a threat to their populations. In the study area, *B. andrewsi* hibernates from late October to mid-March and breeds in late March, while *R. chensinensis* migrates to breeding pools in early March and begins to hibernate in late September (Fei et al., 2009).

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During the migration season, the species cross the road to reach the breeding ponds, hibernation sites or foraging sites. Road mortality may occur during this period. Therefore, *B. andrewsi* and *R. chensinensis* were chosen as sample animals.

2.5 Road-kill surveys

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Road-kill surveys were randomly conducted on seven consecutive days per month in the Wanglang NNR, considering that most carcasses probably persist on the road for 24 h (Santos et al., 2011; Brzezinski et al., 2012). We conducted surveys for a total of 77 days over eleven months. The investigation lasted from April to October in 2017 and during June and August in 2015 and 2016. Road monitoring was conducted by two observers that walked from 8:00-11:30am. Both sides of the road were carefully checked to avoid underestimation of road mortality. All carcasses of amphibians were counted, identified and marked with white foam tape to avoid being double-counted. Geographic coordinates were obtained by a handheld GPS (60), and carcasses were photographed with a Sony DSC-RX100.

Before conducting the road-kill monitoring, we captured and marked toads (*B. andrewsi*) with passive integrated transponder (PIT) tags in April 2017 from the sixth breeding pool (104.15467 E, 32.91746 N), which is located near the Bao-zi Gou conservation station. We intended to identify the marked breeding individuals in the subsequent road-kill study and explore the effects of roads on the population structure of *B. andrewsi* from April to October. In this study, all marked individuals were released to the breeding pool after sampling.

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2.6 Data analyses

Kruskal-Wallis tests were used to determine the differences in road casualties over three years (including only June and August). To determine the fine-scale spatial distribution of amphibian road-kill, the 10 kilometres road was divided into a grid of 0.004 degree boxes, and each box was approximately 445 square metres (21×21 metres). The number of deaths was counted in each box to identify the road-kill hotspots. The environmental factors related to amphibian road-kill included mean daytime temperature (DT, °C), mean night temperature (NT, °C), mean daytime humidity (DH, %) and mean night humidity (NH, %) (day was defined as the time between sunrise and sunset, while night was defined as the time between sunset and sunrise) (https://avcams.faa.gov/sunrise_sunset.php). The data were obtained by a TP-2200 temperature and humidity data logger. We obtained daily precipitation (DP, millimetres) from the meteorological station. To calculate the mean daily traffic volume (MDT) and mean nightly traffic volume (MNT), we checked the vehicle volume from a vehicle monitoring device in the Wanglang NNR.

A variance inflation factor (VIF) was used to determine the factors that were collinear and should be removed before subsequent analyses (Kutner et al., 2004). We defined factors as highly correlated if the VIF was greater than 4, and we removed one of the two tested factors. To identify a set of environmental factors that did not have collinearity, we recalculated the VIF values and repeated the process until all VIF values were less than 4. We began with a generalized linear model (GLM) using a Poisson distribution. Therefore, we needed to check if over-dispersion existed in the counts of road kill. We conducted a Chi-squared test to compare the death data with the standard Poisson distribution with the same mean. We also tested for goodness-of-fit of the model with a chi-square test based on the residual deviance and degrees of freedom. After that, we built a GLM by using the factors without multicollinearity. A GLM was performed to determine the environmental factors that were related to the daily road-kill events.

To identify the optimal model, we used a stepwise backward selection method using Akaike information criterion (AIC) (Akaike, 1974; Sakamoto et al., 1986). We ranked the models separately based on AIC and then determined the best model of all submodels using their AIC values. All analyses were performed in the R statistical environment version 3.4.2 (R Development Core Team 2016).

3. Results

3.1 Species of amphibian road kill

A total of 298 individuals were examined during the survey, including 254 toads (*B. andrewsi*) and 44 frogs (*R. chensinensis*). *B. andrewsi* was the most common amphibian species killed on the road, accounting for 85.2%, followed by *R. chensinensis* (14.8%).

3.2 Temporal patterns of road kill

We conducted a total of 49 road surveys over 490 kilometres from April to October of 2017 and found 88 instances of amphibian road-kill. The number of amphibian road-kill events from April to October in 2017 was not homogenous. The peak amphibian road-kill rate was concentrated in April, when amphibians were observed to move from the breeding pools to the adjacent forest or overwintering sites. By contrast, October exhibited the lowest road-kill rates. The road-kill rate of *B. andrewsi* was the highest in June and July. However, *R. chensinensis* road kill was highest in April, but there were few road-kill events recorded during the surveys during other months (Figure 2).

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Kruskal-Wallis tests showed no differences in mortality within years for both species, *B. andrewsi*: $x^2 = 4.973$, df = 2, P = 0.083 and *R. chensinensis*: $x^2 = 5.571$, df = 2, P = 0.062. However, the results showed downward trends in the mortalities of *B. andrewsi* and *R. chensinensis* in June and August over three years. *B. andrewsi* was recorded with an average rate of 0.51 ± 0.12 individual/kilometre/day, and *R. chensinensis* was recorded with an average rate of 0.07 ± 0.02 individual/kilometre/day (Figure 3).

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3.3 Spatial patterns of road-kill

We drew the ‘heatmap’ according to the road-kill data that were collected over six months (June and August from 2015 to 2017). The spatial distribution of amphibian road-kill was not randomly distributed on the road; namely, we found obvious spatial pattern of road-kill aggregations. There were hotspots that were concentrated in specific road locations with high numbers of road killed amphibian. In our study, hotspots were defined as boxes with more than 17 road-kill events, and these hotspots are shown in red on the map. We finally identified two hotspots, which were both near breeding pools (small blue points) along the 10 kilometres survey route (Figure 4). The hotspots were approximately 500 metres away from the different

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3.4 Driving factors for road-kill

Data were recorded from April to October in 2017 and were used to examine whether road-kill events were associated with environmental factors. The results showed that the mean night temperature had the highest VIF value (VIF = 12.0241), and the other environmental factors had values less than 4. Therefore, the mean night temperature was removed from GLM analyse. In general, the Poisson distribution can be used for fitting count data. We found that the daily road-kill events did not conform to the Poisson distribution, as the variance exceeded the mean. Thus, a negative binomial distribution was used to address the over-dispersion of the count data (White & Bennetts, 1996). Using the step-AIC function in R package MASS, we computed the effects of the environmental factors by comparing the AIC values (Burnham & Anderson 2002). The best model included the mean night traffic and mean daytime humidity (Table 1). The GLM showed that the number of amphibian road-kill events was positively related to mean night traffic volume (Table 2).

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3.5 Road effects on the population

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We calculated whether road mortality posed a substantial risk to the *B. andrewsi* population. During the breeding time, 68 toads were captured by hand and marked with PIT tags in April 2017. Some amphibian carcasses were detected with the PIT-tag reader from April to October. Finally, we recorded 4 tagged toads that were killed by road traffic along the road, and these toads were identified as breeding individuals. As the sampling intervals were seven consecutive days per month, these numbers are certainly underestimates of the real mortality.

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4. Discussion

4.1 Magnitude of amphibian road kill

Actual road mortality is often underestimated in studies on amphibian road-kill events (Gibbs & Shriver, 2005; Langen et al. 2007; Elzanowski et al., 2009; Coelho et al., 2012). In our study over an 11-month period from June 2015 to October 2017, we conducted road-kill monitoring surveys over 77 days and collected only 298 road-killed individuals along the 10 kilometres road in the Wanglang NNR. This low number of deaths might be due to uncontrollable factors. For example, we witnessed that carcasses were eaten by scavengers, such as crows, foxes and leopard cats, which may reduce the number of detected amphibian road kills. In addition, other factors also had effects on the accuracy of road-kill estimations, including weather, traffic volume and research approaches (Santos et al., 2011; Santos et al., 2016). For instance, the weather affected the accuracy of the road-kill counts because it was difficult to detect the carcasses during heavy rain (Guinard et al., 2012; Beebee, 2013). Dead amphibians became nearly completely disintegrated after traffic passed over them (Goldingay & Taylor, 2006). Additionally, high traffic volume could decrease the accuracy of road-kill estimations, such as estimations of *Pseudacris crucifer*, which may be explained by two reasons. *P. crucifer* is not willing to cross a road surface when the traffic volume is too heavy, and heavy traffic can also result in rapid decomposition of the carcasses (Mazerolle, 2004). Research approaches also play an important role in amphibian road kill surveys. Counting while walking is more effective than counting while driving since driving may cause more missed carcasses along the road (Orlowski, 2007; Elzanowski et al., 2009); However, driving can cover a larger expanse of survey routes and save time (Langen et al., 2007).

Our results suggested that *B. andrewsi* and *R. chensinensis* suffered from serious damages due to road traffic in the Wanglang NNR. Toads were more vulnerable to traffic than frogs. The differences in road mortality between the two species might be explained by several factors, including the velocity of the species, detection rate, life-history traits and species abundances. First, *B. andrewsi* has a lower velocity than *R. chensinensis*. Hence, *R. chensinensis* has more chances to escape collisions with cars. In contrast, *B. andrewsi* is much more vulnerable to road mortality due to slow movements. The low detectability of *R. chensinensis* road kill could be due to its small body size. Small corpses were rapidly disintegrated by scavengers or vehicles. This condition makes it more likely to underestimate the actual number of deaths

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(Santos et al. 2011). The post-breeding movements of *B. andrewsi* and *R. chensinensis* were quite different, as *B. andrewsi* would stay around the breeding pools or forests, which may have increased the probability of encountering a road surface, thus increasing the road mortality rate. *R. chensinensis* may stop over for several days to recuperate and then migrate to the mountains for foraging. Furthermore, species abundances also influenced the level of road kill (Goncalves et al., 2018). Based on amphibian monitoring programmes conducted in the Wangling NNR over three years, we found that there were more *B. andrewsi* than *R. chensinensis*.

4.2 The composition of amphibian road kills

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The amphibians that constituted the road killed *B. andrewsi* and *R. chensinensis*. We recorded 0.18 amphibian road-kills per kilometre per year. However, Coelho et al. (2012) conducted a study in the Atlantic Forest and estimated 3.4 amphibian road-kills per kilometre per year, which represents higher amphibian road mortality.

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4.3 Road-kill hot moments

Our results showed that the hot moments of amphibian road kill were mainly concentrated in April. These hot moments were highly related to the life-history, phenology, and particularly migration of the species between breeding sites and hibernation sites. Other studies identified similar results and found that amphibians suffered from higher road mortality during the breeding season (Santos et al., 2007; Hartel et al., 2009). Orlowski (2007) also detected that amphibians exhibited a road-kill peak in April in Lower Silesia, Poland. In addition, *B. andrewsi* achieved the peak rates of road-kill in June and July, possibly because the species moves to foraging areas, which increases road mortality. However, the fewest road-kill instances were recorded in October, probably because *B. andrewsi* and *R. chensinensis* began to hibernate at that time, resulting in fewer records of amphibian road kill. Most amphibians exhibited low mortality rates in the winter due to hibernation (Seo et al., 2015). Overall, the temporal distribution of amphibian road-kills exhibited close relationships with their breeding season and rainy events (Carvalho & Mira, 2011).

4.4 Road-kill hotspots

Our results showed that amphibian road-kill hotspots that occurred along the road were spatially clustered. Previous studies also demonstrated that the distribution of amphibian road-kill events was non-random along the surveyed roads (Sillero, 2008; Langen et al., 2009; Coelho et al., 2012; Seo et al., 2015). Road-kill hotspots were predominantly located in the road 8 kilometres from the start of the survey route (from the Mu-yang Chang conservation station), and these hotspots were associated with breeding pools. A previous study found that amphibians often used the same migratory pathways to cross the road (Santos et al., 2007), which would increase the probability of road mortality due to encounters with vehicles. Amphibians were more vulnerable to traffic mortality near water bodies (Orlowski, 2007; Seo et al., 2015; D'Amico et al., 2015).

4.5 The reason for road-kill

Our findings indicated that night traffic was a fatal factor that contributed to amphibian road kill, as the number of dead amphibians increased with the increase in night traffic volume. However, we also found that the number of dead amphibians maybe is associated with the mean daytime humidity since AIC values were similar between models MNT+DH and DH. Amphibian roaddkills often have a positive relation to humidity, in particular during migration, amphibians have suffered higher road mortality on humid environment (Duellman & Trueb, 1986; Garriga et al., 2017). Amphibian road-kill rates were prone to increases as the traffic volume increased (Fahrig et al., 1995; Hels & Buchwald, 2001). Previous studies showed that the mean night traffic affected amphibian road mortality (Sutherland et al., 2010; Coelho et al., 2012). However, night traffic has rarely been specifically proposed to explain fatal collisions. In addition, previous studies did not provide detailed traffic volumes in a day. Little information had been provided on the traffic time range in a day that has the highest road-kill ratio. However, most amphibian species are nocturnal, and amphibian collision events often occur at night (Hels & Buchwald, 2001). Thus, our study has considered both the daytime and nighttime traffic flows instead of the mean night traffic, and the traffic at night was the factor that determined amphibian road-kill. Therefore, we should restrict the volume of tourist traffic at night to efficiently reduce road mortality, and this measure will contribute to better ecotourism.

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5. Conclusion

Amphibian road kill was temporally and spatially aggregated along the road in the Wanglang NNR. Road-kill hot moments were concentrated in April, June and July. Road-kill hotspots were concentrated in the road section near the breeding pools. Based on our findings, measures of both hot moments and hotspots have to be taken to reduce the road-kills in the Wanglang NNR. First, based on road-kill hot moments, we should apply temporary traffic restraints at night during the breeding season and from June-July. Second, based on road-kill hotspots, amphibian culverts should be established in the areas of breeding pools adjacent to roads, and fences should be installed to guide amphibians into the culverts.

Acknowledgements

We are grateful to Qiang Dai and Youhua Chen for comments on the manuscript. We also thank Hongliang Bo and Yuanbin Zhang for providing advice for the work.

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