Factors contributing to amphibian road mortality in a wetland

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Abstract To understand road characteristics and landscape features associated with high road mortality of amphibians in Zoige Wetland National Nature Reserve, we surveyed road mortality along four major roads after rainfall in May and September 2007. Road mortality of three species, Rana kukunoris, Nanorana pleskei and Bufo minshanicus, was surveyed across 225 transects (115 in May and 110 in September). Transects were 100 m long and repeated every two kilometers along the four major roads. We used model averaging to assess factors that might determine amphibian road mortality. We recorded an average of 24.6 amphibian road mortalities per kilometer in May and 19.2 in September. Among road characteristics, road width was positively associated with road mortality for R. kukunori and B. minshanicus. Traffic volume also increased the road mortality of B. minshanicus in September. Of the landscape features measured, area proportions of three types of grassland (wet, mesic and dry) within 1 km of the roads, particularly that of wet grassland, significantly increased road mortality for R. kukunori and total mortality across all three species. To most effectively reduce road mortality of amphibians in the Zoige wetlands, we suggest better road design such as avoiding wet grasslands, minimizing road width, underground passes and traffic control measures. The implementation of public transit in the area would reduce traffic volume, and hence mortality [Current Zoology 57 (6): 768–774, 2011].

Keywords Amphibian, Road mortality, Zoige Wetland, Rana kukunoris, Nanorana pleskei, Bufo minshanicus

The impact of roads on biodiversity has attracted much attention (Carr and Fahrig, 2001; Coffin, 2007; Fahrig and Rytwinski, 2009; Trombulak and Frissell, 2000). Wild animal mortality due to road kill has exceeded that caused by human hunting and poaching in recent years (Forman and Alexander, 1998). Amphibians are particularly vulnerable to traffic mortality, because they cross roads slowly and are not easily noticed and avoided by drivers (Hels and Buchwald, 2001; Vijayakumar and Vasudevan, 2001). In some areas, mortality of amphibians due to road kill has become a threat to the stability of local populations (Gibbs and Shriver, 2005).

Previous studies found that amphibian road mortality was related to the types of habitat near roads and the amount of road traffic (Clevenger et al., 2003; Mazerolle, 2004). A better understanding of road characteristics and landscape features associated with high road mortality is critical in future road design where conservation of amphibians is a priority. By avoiding the construction of roads in areas with high amphibian densities and migration routes, we can reduce mortality associated with roads and better protect amphibian populations.

The Zoige Wetland is located in the northeastern corner of the Qinghai-Tibet Plateau. It is of global wetland biodiversity conservation significance, but is vulnerable to disturbance (Yang, 1999; McNamee, 20031; Yan and Wu, 2005). Three amphibian species, Rana kukunoris, Nanorana pleskei and Bufo minshanicus are present in Zoige Wetland. With the development of the local economy in recent years, both the number of major roads and road density in the wetland have dramatically increased. However, the impact of roads on wildlife populations in the region remains unknown. Here we quantify the extent of amphibian road mortality in Zoige Wetland and determine which road and landscape features in areas surrounding roads are associated with high levels of amphibian mortality.

1 Materials and Methods

1.1 Survey area

We surveyed four major roads, G 213, S 209, Tang-Hei County Road and Hei-Re County Road, surrounding Zoige Wetland National Nature Reserve. All

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reserves a total area of 1666 km$^2$ and is located in Qiang Autonomous Prefecture, Sichuan Province. The region of 3400–3700 m, within Zoige County, Aba Tibetan and Qiang Autonomous Prefecture, Sichuan Province. The reserve covers a total area of 1666 km$^2$ and is located in the core of Zoige Wetland. The wetland is a herbaceous marsh and is also an important water resource upstream of the Yellow River.

### 1.2 Study animals

All three species of amphibians inhabiting Zoige Wetland are endemic to the Qinghai-Tibet Plateau. The conservation status of *R. kukunoris* and *B. minshanicus* are “Least Concern” while that of *N. pleskei* is “Near Threatened” according to the IUCN Redlist (IUCN 2011). Previous surveys showed declining populations for all three species in Zoige Wetland, especially for *N. pleskei* (Fellers et al., 2003). All three species live in alpine meadow, marshland and grassland habitat near small water bodies such as pools and streams; however, *B. minshanicus* can disperse farther away from these water bodies than *R. kukunoris* and *N. pleskei* (Dai et al., 2005a; Dai et al., 2005b). Habitat loss and degradation caused by overgrazing, road construction, and possibly climate change are the most important threats to populations of these three species in the Zoige Wetland (Dai et al., 2005a). In the wetland, *R. kukunoris* and *N. pleskei* hibernate from October to late April and breed in May (Fei et al., 2009a, b). *B. minshanicus* hibernates from October and begins to breed in late March to May (Fei et al., 2009a). Road mortality surveys were carried out in the breeding season (May) and before hibernation (September), when migration was common and road mortality was likely at its peak.

### 1.3 Survey methods

Considering seasonal variation in amphibian activity and mortality, we surveyed the roads in May and September of 2007. We surveyed 115 transects and 11.5 km of road in May and 110 transects and 11 km of road in September along the four roads. Each road was divided into 2-km road segments, within which there was no major exit. We selected a single 100-m transect at the beginning of each 2-km road segment to sample amphibian road-kills. Because there were no reliable landmarks along the roads, the distance of 2 km was measured using a car odometer. Location of each transect was recorded at its mid-point with a GPS. Two people collected and recorded all dead amphibians along 100-m transects in approximately 5–10 min. We only surveyed roads in the morning between 8:30 and noon when it rained the previous night and continued into the morning for two reasons. First, amphibians are more active during rainy days and after rainfall when the ground is still wet, thus they are more vulnerable to road kill during this time (Cairo and Zalba, 2007; Qi et al., 2007). Second, these roads are swept and cleaned daily by workers in the morning except on rainy days. Our schedule ensured that all road kills within the last 24 hours would be recorded were counted, except for those removed by scavengers (Enge and Wood, 2002). If it became sunny and workers began to sweep the roads, we terminated our survey for the day. To make our data comparable to previous studies, we expressed road mortality density in the form of road mortality/surveyed distance (km).

We used vehicle encounter rate to represent traffic volume. We drove between transects, and walked within each transect. During the process, we recorded every motor vehicle (including motorcycles) encountered. We ensured that we did not count a vehicle more than once by driving slowly (about 40 km/h) and allowing other vehicles to pass. We defined the vehicle encounter rate as the total number of vehicles recorded divided by the survey time. Two motorcycles were considered the equivalent of one vehicle. All other motor vehicles were treated equally. We did not survey traffic volume at night. It was assumed that road traffic volume at night was low and was correlated with daytime traffic. By so doing, we ensured a close linear correlation between vehicle encounter rate and total traffic volume within each road section. Road mortality and vehicle encounter rate of each road were surveyed only once.

Habitat types were interpreted from satellite images. These images were taken from the Landsat in September 2007 with a resolution of 30 m. Studies investigating multiple spatial scales have found that landscape types within 1 km contribute most to predict the distribution of amphibians (Pope et al., 2000; Homan et al., 2004). The area of five habitat types, bare land, water body, wet grassland, mesic grassland, and dry grassland, within 1 km surrounding each transect were measured. Wet grassland is marsh or vegetation covered area patterned with a seasonal or annual water logged period, in which small pools and streams are common. Dry grassland is characterized by sparse plants and relatively dry soil. Mesic grassland is distributed between the two mentioned grassland types with relatively moderate soil moisture and dominated with mesic grasses and sedges. A relationship between distribution of road mortality and surrounding habitat types was examined.
1.4 Data analysis

Based on previous research on habitat selection for our focal species (Dai et al., 2005a) and influence of road width and traffic volume on road mortality (Fahrig et al., 1995; Forman and Alexander, 1998), we tested 18 ecologically plausible logistic regression models (Supplementary material*) to evaluate the relationships between road mortality presence probability and road width, vehicle encounter rate and the proportion of habitat types within 1 km of each transect (Burnham and Anderson, 2002; Johnson and Omland, 2004). Road mortality presence of total amphibian, R. kukunoris and B. minshanicus were analyzed separately for May and September. Presence of N. pleskei mortality were not analyzed separately because too few transects with dead N. pleskei were found in May (eight transects) and in September (five transects). The proportion of habitat type is represented as a percentage. The percentage data were square-root transformed, to increase the influence of some habitat types when rare. Furthermore, as the percentages of all five habitat types summed to one, and therefore were not independent from each other, we used percentages of four of the five habitat types (water body, wet grassland, mesic grassland, and dry grassland) for analysis.

Models were ranked according to AICc (Akaike's information criterion corrected for small sample size) (Burnham and Anderson, 2002). Since no single model was significantly better than others according to AICc, i.e. Akaike difference > 2 or Akaike weight > 0.9, model averaging estimate was used based on all candidate models (Anderson et al., 2001; Burnham and Anderson, 2002). Estimated coefficients of each variable were assessed with Aikake weights. Sum of Aikake weights (∑ωi) over all possible models that include a specific predictor was used to assess the relative importance of that predictor variable in explaining variation of the overall model, which provides a more robust assessment of variable importance, relative to single-model approaches (Burnham and Anderson 2002; Anderson 2008). Model analyses and averaging were conducted with the “AICcmodavg” package (Mazerolle, 2011) in R (R Development Core Team, 2011).

2 Results

2.1 Composition and seasonal variation in road mortality

In May a total of 283 amphibians road mortalities were recorded in the 115 transects surveyed (Table 1). Road mortalities of amphibians were recorded in 34 (29.6% of total) transects, with an average of 24.6 ± 6.7 (SE) road mortalities/km. R. kukunoris was recorded in 20 (17.4% of total) transects, with an average of 15.3 ± 5.7 (SE) road mortalities/km. B. minshanicus was recorded in 8 (7.0% of total) transects with an average of 4.0 ± 2.0 (SE) road mortalities/km. N. pleskei was recorded in 19 (16.5% of total) transects, with an average of 5.3 ± 1.8(SE) road mortalities/km. Among the killed amphibians, R. kukunoris was the most common and account for 89.1% (176) of kills, followed by B. minshanicus, which account for 6.6% (61) of kills. N. pleskei was the least common and accounted for 4.3% (46) of total road mortalities.

In September a total of 211 amphibians road mortalities were recorded in the 110 transects surveyed (Table 1). Road mortalities were recorded in 34 (29.6% of total) transects with an average of 19.2 ± 0.28 (SE) road mortalities/km. R. kukunoris was recorded in 13 (11.8% of total) transects with an average of 1.9 ± 0.03 (SE) road mortalities/km. N. pleskei was found in 5 (4.6% of total)

<table>
<thead>
<tr>
<th>Description</th>
<th>Starting point - destination</th>
<th>Length (km)</th>
<th>Width (m)</th>
<th>Number of transects (May/Sept)</th>
<th>Road mortality (May/Sept)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R. kukunoris</td>
</tr>
<tr>
<td>G 213</td>
<td>Langmusi Tunnel-Dazhasi Town</td>
<td>54</td>
<td>9</td>
<td>30/28</td>
<td>54/6</td>
</tr>
<tr>
<td>S 209</td>
<td>Dazhasi Town-Tangke Town</td>
<td>66</td>
<td>7</td>
<td>33/29</td>
<td>109/2</td>
</tr>
<tr>
<td>Tang-Hei County Road</td>
<td>Tangke Town-Heihe Pasture</td>
<td>48</td>
<td>7</td>
<td>40/41</td>
<td>1/2</td>
</tr>
<tr>
<td>Hei-Re County Road</td>
<td>Heihe Pasture-Reerba</td>
<td>66</td>
<td>7</td>
<td>12/12</td>
<td>12/4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>234</td>
<td>115/110</td>
<td>174/14</td>
<td>61/118</td>
</tr>
</tbody>
</table>

* See the journal webpage.
transects with an average of $0.08 \pm 0.04$ (SE) road mortalities/km. *B. minshanicus* was found in 56 (50.9% of total) transects with an average of $1.07 \pm 0.27$ (SE) road mortalities/km. Among the killed amphibians, *B. minshanicus* was the most common and accounted for 62.2% (118) of total road mortalities, followed by *R. kukunoris*, which accounted for 21.6% (14) of total mortalities. *N. pleskei* was the least common and accounted for 16.3% (9) of the total mortalities.

### 2.2 Spatial distribution of road mortality and determining factors

In May a total of 10, 11, 4 and 3 transects recorded amphibian road mortality from G 213, S 209, Hei-Re road and Tang-Hei road, respectively. The distributions of road mortality of each species among the four roads were similar with that of total amphibian road mortality (Fig. 2). In September a total of 16, 20, 6 and 18 transects recorded amphibians road mortality in G 213, S 209, Hei-Re road and Tang-Hei road, respectively (Fig. 2).

Model averaging analysis suggested that both road width and surrounding habitat composition were important variables in predicting the overall road mortality of amphibians in May (Table 2, Fig. 2). The proportion of wet grassland area within 1 km best predicted road mortality ($\sum \omega_i = 0.997$). It was positively associated with the presence of amphibian road mortality. In addition to wet grassland area, road width showed great contribution to predict road mortality ($\sum \omega_i = 0.997$) with a significant positive influence. Proportion of mesic grassland and dry grassland also was positively associated with amphibian road mortality, but with a lesser contribution ($\sum \omega_i = 0.937$ and 0.886 respectively).

In September the proportion of grassland within 1 km, including wet grassland, mesic grassland and dry grassland, best predicted road mortality of amphibians with $\sum \omega_i = 0.922, 0.919$ and 0.866 respectively (Table 2, Fig. 2). A larger proportion of the three types of grassland surrounding the road increased the presence probability of amphibian road mortality.

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**Fig. 1** Percent of sample transects with amphibian road mortalities

**Fig. 2** Relative importance (sum of Akaike weights) of variables in logistic models predicting the road mortalities of amphibians (white bars), *R. kukunoris* (grey bars) and *B. minshanicus* (black bars)

Traffic = Vehicle encounter rate; Width = Road width; Mesic = percentage of mesic grassland area within 1 km of transect; Wet = percentage of wet grassland area within 1 km of transect; Dry = percentage of dry grassland area within 1 km of transects; Water = percentage of water body surface area within 1 km of transect.
Table 2  Model averaged coefficients, unconditional standard errors for logistic regression models predicting presence of road mortalities

<table>
<thead>
<tr>
<th>Variable</th>
<th>May Coefficient</th>
<th>May Unconditional standard error</th>
<th>September Coefficient</th>
<th>September Unconditional standard error</th>
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<td>Amphibians</td>
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<td>0.04</td>
<td>0.03</td>
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<tr>
<td></td>
<td>Width 1.33*</td>
<td>0.36</td>
<td>0.52</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Mesic 9.75*</td>
<td>3.81</td>
<td>8.06*</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>Wet 7.95*</td>
<td>2.23</td>
<td>4.96*</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>Dry 9.92*</td>
<td>4.00</td>
<td>8.35*</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td>Water −9.60</td>
<td>7.69</td>
<td>1.80</td>
<td>2.49</td>
</tr>
<tr>
<td>R. kukunoris</td>
<td>Traffic 0.02</td>
<td>0.05</td>
<td>−0.08</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Width 0.93*</td>
<td>0.35</td>
<td>0.85*</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Mesic 8.78</td>
<td>4.55</td>
<td>6.93</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>Wet 7.69*</td>
<td>2.68</td>
<td>7.19*</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>Dry 10.98*</td>
<td>4.70</td>
<td>10.47</td>
<td>5.63</td>
</tr>
<tr>
<td></td>
<td>Water −8.88</td>
<td>10.11</td>
<td>−15.56</td>
<td>18.38</td>
</tr>
<tr>
<td>B. minshanicus</td>
<td>Traffic 0.06</td>
<td>0.06</td>
<td>0.06*</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Width 0.89*</td>
<td>0.37</td>
<td>0.36</td>
<td>0.31</td>
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<tr>
<td></td>
<td>Mesic 5.90</td>
<td>4.49</td>
<td>5.80</td>
<td>3.17</td>
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<td>Wet 3.74</td>
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<td></td>
<td>Dry 7.08</td>
<td>4.39</td>
<td>6.24*</td>
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<tr>
<td></td>
<td>Water −4.97</td>
<td>7.30</td>
<td>2.66</td>
<td>2.54</td>
</tr>
</tbody>
</table>

*Traffic= Vehicle encounter rate; Width = Road width; Mesic = percentage of mesic grassland area within 1 km of transect; Wet = percentage of wet grassland area within 1 km of transect; Dry = percentage of dry grassland area within 1 km of transects; water = percentage of water body surface area within 1 km of transect. The estimated coefficient is different from 0 at significance level of 0.95.

In May and September the proportion of wet grassland area within 1 km best predicted road mortality for *R. kukunoris* (May: $\Sigma \omega_i = 0.984$, September: $\Sigma \omega_i = 0.923$), followed by road width (May: $\Sigma \omega_i = 0.883$, September: $\Sigma \omega_i = 0.756$) (Table 2, Fig. 2). Both the proportion of wet grassland and road width were positively associated with road mortality for *R. kukunoris*. Road features such as road width and traffic volume are important predictors for the presence probability of *B. minshanicus* road mortality (Table 2, Fig. 2). In May road width best predicted the presence of *B. minshanicus* road mortality ($\Sigma \omega_i = 0.819$) with a significant positive influence; traffic volume best predicted the presence of *B. minshanicus* road mortality ($\Sigma \omega_i = 0.791$) with a significant positive influence in September.

3 Discussion

An average of 24.6 amphibian road mortalities/km was found in May and 19.2 in September, after rainfall. It is still unknown whether road mortality in Zoige Wetland causes population decline for these species. Actual road mortality could be higher than recorded because a substantial proportion of carcasses could have been removed by scavengers during the night (Enge and Wood, 2002). Meanwhile, road mortality in days without rain is likely much lower than we reported because amphibians are more vulnerable to road mortality in rain (Cairo and Zalba, 2007).

Comparison of amphibian road mortality between studies is often difficult because of different survey methods and local population conditions (Langen et al., 2007; Elzanowski et al., 2009). The road mortality density of amphibians in Zoige Wetland was similar to that in a national park in eastern Canada (125.4 individuals per night on 20 km road), which did not cause population decline (Mazerolle, 2004). Surveys on a road near Lake Erie (Canada) also showed a similar road mortality density of 11.65 individuals/km/day (Ashley and Robinson, 1996). Other surveys reveal very different results: a low amphibian road mortality on a Mediterranean farmland road (0.808 individuals/km) (Carvalho and Mira, 2011), high mortality density of 84.4 individuals/night on 200 m of a coast road in Australia (Goldingay and Taylor, 2006) and 174.7 individuals/km...
on rural highway in northern New York, USA (Langen et al., 2007).

Model averaging assessment showed that wide roads resulted in more road mortality of amphibians than narrow roads. This is intuitive because it takes more time for an amphibian to cross a wide road, increasing the probability of it being crushed by vehicles (Fahrig et al., 1995; Mazerolle, 2004). High traffic volume only showed a significant positive effect on \( B. \) minshanicus. This could possibly be due to the behavioral differences between species on the road when confronted by approaching vehicles (Mazerolle et al., 2005).

Wet grassland is the most important habitat for amphibians in the Zoige Wetland. Our previous studies show that wet grassland, together with small water bodies (streams, small pools) within it, had the highest density of amphibians among several habitat types across the wetland (Dai et al., 2005a; Dai et al., 2005b). Previous work also suggested that highest road mortality often occurs in areas with the best habitat for amphibians (Malo et al., 2004). Therefore, it is not surprising that the highest road mortality of amphibians in Zoige Wetland occurs in road sections with a high proportion of wet grassland within 1 km. High population density and frequent activity of these amphibians in such areas probably contributes to the large number of road mortalities. The proportion of water surface area had minor impact on road mortality of amphibians. This is likely because the density of amphibians around large water bodies such as rivers and lakes was actually very low at Zoige Wetland (Dai et al., 2005a). The surrounding habitat type was not as important in predicting the presence probability of \( B. \) minshanicus road mortality as it was in predicting that of \( R. \) kukunoris. This is likely because the distribution of \( B. \) minshanicus is less restricted to wet grassland than \( R. \) kukunoris (Dai et al., 2005a; Dai et al., 2005b).

In summary, road width, traffic volume and habitat surrounding roads have a major impact on the road mortality of amphibians. Using landscape analysis to select appropriate road routes while avoiding a range of important habitats for wildlife is a practical measure in road design. Furthermore, minimizing road width is not only superior economically but also conservation wise. Constructing underground passes and scientific traffic management that may minimize the traffic volume will alleviate the mortality of wild animals caused by roads. Encouraging the use of public transport instead of driving motorcycles or cars can decrease traffic volume and thus decrease the impact of traffic on wildlife. In addition, it may be worth considering removing or reducing unimportant wet grasslands near roads when they are functioning as an ecological trap causing greater amphibian mortality than the benefits they provide to local amphibian populations.

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