Patterns of amphibian road-kills in the Vendée region of Western France

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Amphibian road-kills were monitored over a six-year period (2005–2010) on a series of low traffic volume roads in Vendée, Western France. Most road-kill was found in the common toad *Bufo bufo* (39% of all mortalities) and agile frog *Rana dalmatina* (25.4%). Three species of urodeles constituted 26% of the sample. Lowest road-kill was found in *Pelophylax lessonae* (4%), which was attributed to a sedentary lifestyle around ponds. Road-kill had a strong temporal aspect and was associated mostly with migratory movements. Significantly higher than expected road-kill was found on low traffic roads bordered by woodland and/or wetlands in *R. dalmatina*, *Lissotriton helveticus* and *B. bufo* and in urban areas greater than expected in *Triturus marmoratus*, *P. lessonae* and *B. bufo*. Less than expected road-kill was found next to monocultures in all species tested. Road-kill was more numerous following rainfall but during dry weather relatively more frequent in *R. dalmatina* and *P. lessonae*. This was attributed to foraging activity in *R. dalmatina* and movement due to pond desiccation during late summer in *P. lessonae*. Synchronized patterns of road-kill were found in three species of urodeles suggesting similar patterns of movement behaviour. Traffic intensity and road-kill on different roads was not correlated and hence traffic volume was not a good predictor of road-kill. However, in a regression analysis, road-kill showed a strong association (r^2=0.87) with extent of migratory distances.

Key words: Amphibians, habitat, movement behaviour, road-kill, traffic volumes

INTRODUCTION

Road traffic and their effects on ecosystems have been identified as contributing factors in the worldwide decline of amphibians (e.g. Beebee & Griffiths, 2005). Amphibian life history characteristics in temperate climates make them particularly susceptible to road-kill, mainly due to seasonal migration between breeding ponds and summer home ranges (Puky, 2006). Outside the migratory season, utilising road surfaces for thermoregulatory or foraging opportunities increases the risk (Hartel et al., 2009). However, life history characteristics may differ between and within regions (Puky, 2006) and hence identifying elevated spatial and temporal risk in road-kill is crucial for effective targeting of mitigation measures. Amphibians frequently undergo annual population fluctuations that may mask road-kill effects (e.g. Kiesecker et al., 2001; Green, 2003) and to minimize this problem a period for road-kill research that lasts at least three years has been proposed (e.g. Sillero, 2008). This study attempts to address population effects by employing a six-year data set.

Road-kill research in amphibians has primarily focused on medium and high traffic roads (e.g. Lodé, 2000) but low volume roads in Europe have shown substantial increases in traffic in recent years (Jaarsma &Willems, 2002) and their impact on amphibians is rarely reported. Since amphibian movement is primarily nocturnal when traffic volumes are at their lowest, in theory road-kill risk should be lower than in day-active reptiles and lower with decreasing traffic volumes if the latter is the main determining factor involved. Compared to reptiles amphibian travel speeds are limited (e.g. Avery et al, 1987; Isaac & Gregory, 2007) and they may remain on roads for longer (Hels & Buchwald, 2001) due to, for example, physiological limitations for sustained effort and hence may be at greater risk even if traffic levels are lower during a road crossing.

This paper reports on road-kill on a series of low traffic volume roads bisecting a fragmented landscape in Western France. The primary objective of the study was to test if amphibian life history cycles determine road-kill risk. In theory risk should be lowest when adult amphibians are in their aquatic phase, increase during the terrestrial phase and peak when major migration takes place. The study also sought to examine spatial and habitat-associated road-kill and how changes in road traffic volumes may impact on road-kill (Rowe, 1977; Sutherland et al., 2010).

METHODS

Surveying was carried out from January 2005 to December 2010 on six roads. Figure 1 shows the area (46°27N) with information on key habitats including breeding locations. The relevant parameters recorded were monocultures (40.8% of roadside habitat), woodland/wetlands (35.3%), urban areas (23.9%) and major breeding ponds. Monocultures are transient environments due to crop rotation (e.g. wheat or rape seed oil production) and the presence or absence of grazing animals. Woodlands have

been linked to wetlands due to extensive flooding of wooded areas during the winter months, which persists until around June.

Between 4 and 6 surveys per month at around 4 day intervals were carried out usually by a single observer between 1000–1700 hours on a bicycle at a maximum speed of around 5–10 km/hour. Surveys were spaced 3–4 days apart, normally at mid-week and weekends. Sampling for both road-kills and live animals required between 5–7 hours for the D44, D60 and St Denis-Du-Payré to Chasnais road and around 2 hours for the D949, D127 and Rue de Bourneau. Due to time constraints, the D949, D127 and Rue de Bourneau were surveyed on different days to the D44, D60 and St Denis-Du-Payré to Chasnais road. The roads differ in width from 5 to 7 metres and have one lane in each direction.

The road segments connect the town of Lucon to the village of Chasnais (D949~1.2 km); Chasnais to Lairoux (D44~1.2 km); La Bretonnière-la-Claye to St Denis-Du-Payré (D60~6.1 km); Chasnais to Triaize (D127~1 km); Rue de Bourneau in Chasnais village ~0.5 km. The road running from St Denis-Du-Payré to Chasnais (no designated number or name) originates at Rue de Moulins in the village of St Denis-Du-Payré and runs in the direction of Chasnais for about 6 km.

The climate is mild oceanic (June, July and August monthly mean air temperature=26 °C; November through to February monthly mean=10.2 °C). The period of high precipitation is from October until January, monthly mean=85.7 mm. During summer (June, July and August monthly mean=51.3 mm) all but the largest water bodies dry up.
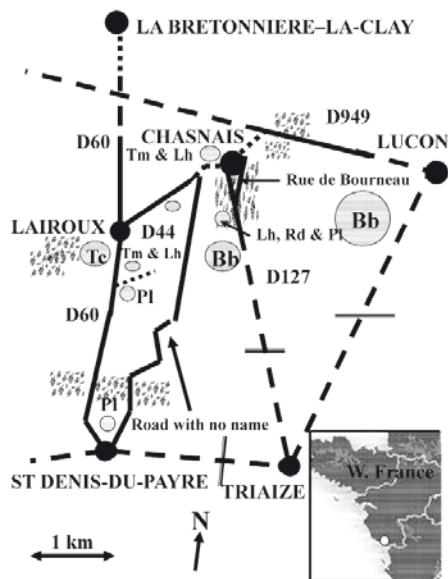

Fig. 1. Map of study locality showing roads surveyed (heavy lines) and their locality in relation to breeding ponds. Broken lines indicate non-surveyed roads and are not to scale when intersected by lines. Patterned areas represent woodland and/or wetlands. Abbreviations are: Pl *P. lessonae*, Tc *T. cristatus*, Tm *T. marmoratus*, Lh *L. helveticus*, Bb *B. bufo*, Rd *R. dalmatina*. White spot on the insert shows the approximate locality of the study site.
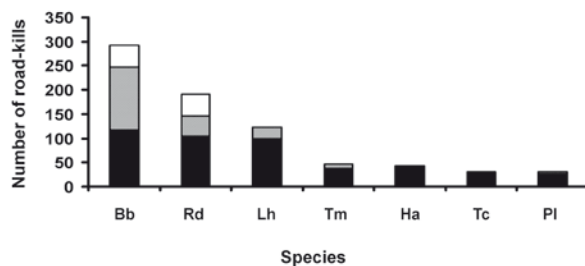

Fig. 2. Cumulative road-kill over the six-year period. On the x-axis Bb = *B .bufo*, Rd = *R. dalmatina*, Lh = *L. helveticus*, Tm = *T. marmoratus*, Ha = *H. arborea*, Tc = *T. cristatus* and Pl = *P. lessonae*. Solid, cross hatched and open areas represent adults, subadults and juveniles respectively. In *B. bufo* solid bars indicate large females, cross hatched adults and open juveniles. Sub-adult numbers in *H. arborea*, *T. cristatus* and *P. lessonae* were too small to illustrate.

Sampling methods

Late evening traffic volume was recorded to match amphibian movement behaviour, which in the study area begins just after dusk peaking between 2300 hours and midnight. Hourly traffic levels were counted for this period once per month on each road and analysis applied to mean values. Surveying for road-killed and live animals was between approximately 1000 and 1800. To gain a basic insight into species presence and their approximate numbers, live surveys were carried out randomly selecting different roadside segments during each road-kill survey. The areas searched were up to 100 m from roadsides, for animals either in ponds, under logs/rocks or that were active. Individuals were captured and measured or, if capture was not possible, simply counted. Since no animals were marked it is not possible to be certain that live animals were counted more than once but the counts were over a wide geographical area and time period and hence double counts should be minimal. Annual and seasonal sampling effort was approximately equal except during periods when snow covered the roads, which was no longer than 2 weeks in any year.

Seven species of amphibians were found as road-kill. The agile frog (*Rana dalmatina*) was the earliest breeder, appearing in ponds and ditches from February until early March. After the reproductive period it forages in woodlands/wetlands but occasionally appears in gardens and fields. Identification of *Pelophylax* (=*Rana*) *lessonae* was visual, from the ARC Internet site sound recordings (http://www.alienencounters.org.uk/marshfrog.html) and from water frog distribution in France (Pagano et al., 2001). It was found around ponds and ditches throughout the summer where it appears to have strong site fidelity unless the pond desiccates in mid to late summer. Migration of the toad *Bufo bufo* took place between October and November for the reproductive period in March. They returned to terrestrial habitats during April where they forage in limited home ranges nocturnally during the summer months (Meek & Jolley, 2006). The tree frog *Hyla arborea* appeared near ponds in
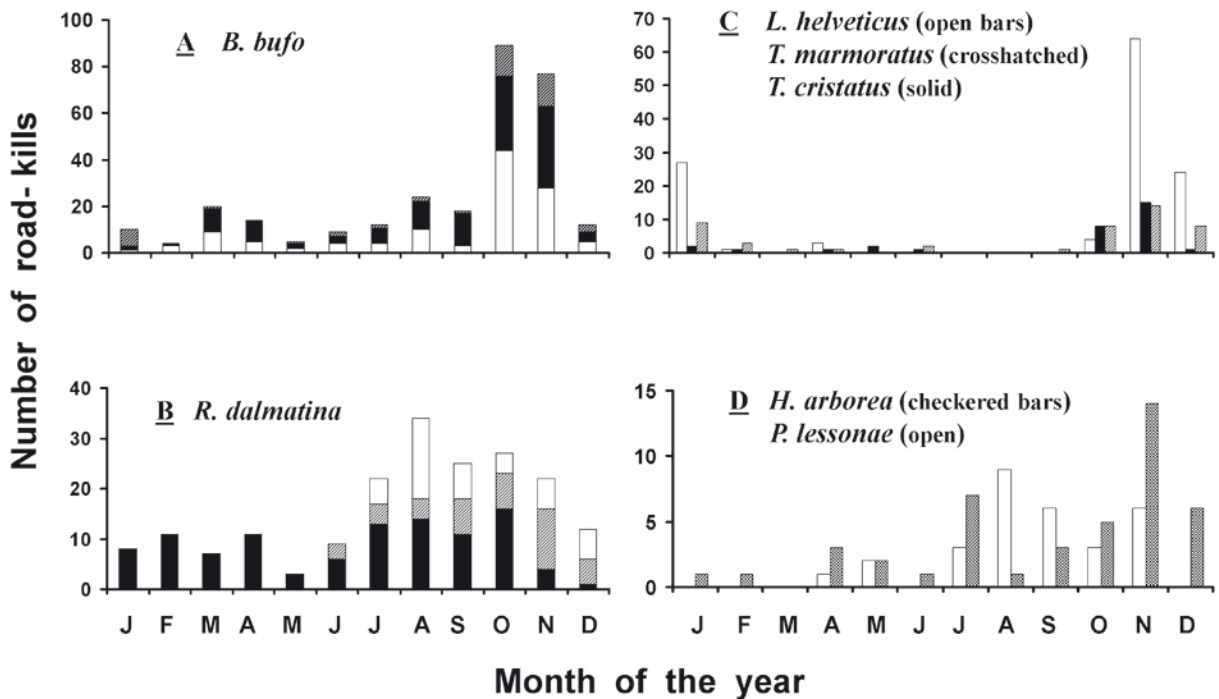
Fig. 3. Cumulative monthly road-kill frequencies over the six-year period indicating autumn and/or late summer road-kill in most species. High summer mortalities beginning in July in *R. dalmatina* are also apparent. Definitions of histograms for *B. bufo* and *R. dalmatina* are as indicated in Fig 2. Keys to histograms in C and D are shown in the graphs. Note differences of scale on y-axis.

March/April with spawn clumps found from late April to June. Calling could be heard remotely from ponds until November. Life history cycles of *Lissotriton* (=*Triturus*) *helveticus*, *Triturus marmoratus* and *T. cristatus* are in general similar (Arnold & Ovenden, 2002). They appeared in ponds between November and January. Reproduction took place from February before dispersing to terrestrial habitats from around late May. During the summer terrestrial phase *T. marmoratus* and *T. cristatus* apparently remain below ground (Jehle, 2000). Fewer live counts were made on *T. cristatus* during both aquatic and terrestrial phases. It may occupy deeper water and remain in the aquatic phase for longer (Frazer, 1983). Following the summer terrestrial phases all species of urodeles migrate and return to aquatic habitats, others may hibernate before entering water (e.g. *H. arborea*, *B. bufo* and *R. dalmatina*); *P. lessonae* hibernates at the bottom of ponds. All species found in the live animal surveys were recorded as road-kill.

Size classes (in mm) were assigned arbitrarily. They were: for *B. bufo*, large females ≥80, adults ≥40–79 and sub-adults/juveniles <40; *R. dalmatina*, adults ≥65; subadults ≥40–64, juveniles <40. In the remaining species only two size classes were recognised. Adult thresholds were: *P. lessonae* ≥50; *H. arborea* ≥30, *T. marmoratus* and *T. cristatus* ≥45 and *L. helveticus* ≥28.

Statistical analysis

All statistical tests were set at the 95% interval and ± values represent standard deviations except in the regression

equation when it is a standard error of the regression coefficient. Inter-specific comparisons in proportions have been made using z-tests and for regularity of monthly or seasonal road-kill χ^2 tests with means treated as the expected or null hypothesis. Regression analysis was applied to total road-kill for each species in relation to their known migratory distances from data in the literature (Heusser, 1968; Simms, 1969; Blab, 1986; Jehle, 2000; Puky, 2006). Determining distances moved by *P. lessonae* was problematical as reports of movement often refer to a single or a few individuals but apparently pond fidelity within season is normal (Sjogren-Gulve, 1998) with a displacement distance of around 150 m (Holenweg et al., 2001); this figure has been arbitrarily employed in the analysis. Regional differences in amphibian movement are known and hence the method only gives approximate results. A test against the theoretical coefficient of 0 in the regression (no effect of migratory distance on road-kill levels) was made using a t-test at n-2 degrees of freedom described by Bailey (1981).

Approximate biomass of road-killed *B. bufo* and *R. dalmatina* were estimated using allometric equations derived from regression analysis of the logarithms of s.v. length and logarithms of body mass of live animals (*B. bufo, n=43* and *R. dalmatina, n=37*). Straight-line s.v. length (in mm) was treated as the independent variable and body mass (in grams) as the dependent variable. This gave for *B. bufo*; body mass=0.0001s.v.length$^{3.05}$ (r^2=0.98) and *R. dalmatina*, body mass=0.00006s.v.length$^{3.09}$ (r^2=0.89).

Road-kill and habitat association

Roadside habitat was estimated by a combination of recording approximate distances in metres on both sides of the road using specific survey points (i.e. buildings, bridges woodland edges etc.) and also the distance-calculating tool on *Google Earth*. When a road-kill was located with similar habitat on both sides a score of 1 was applied; when habitats differed (e.g. woodland on one side, urban on the other) the score was 0.5:0.5. From these numbers percentages of available roadside habitat was calculated and compared with the corresponding numbers of road-kill found alongside habitat type using a one dimensional χ^2 Goodness of Fit test set at the 95% interval. The test evaluates the extent of percent deviation of the observed road-kill counts from the expected based on habitat availability - with the latter entered as decimal proportions. The null hypothesis is true if road-kills are randomly distributed and in statistical agreement with distributions of roadside habitat.

RESULTS

General counts

Twenty-one unidentified anurans (none were *B. bufo*) and 39 urodeles (probably *L. helveticus*) were also found. Identified road-kills totalled 759 of which 559 (73.6%) were anurans (Fig. 2). Taken over the six-year period there was an inter-specific imbalance in casualty counts (χ^2=563.8, *p*<0.0001) with the common toad *B. bufo* (*n*=294; 38.7%) and agile frog *R. dalmatina* (*n*=191; 25.4%) the most frequent. Urodeles formed just over 26% of the total and were mostly *L. helveticus* (*n*=122; 16.1%) with *T. marmoratus* 6.2% and *T. cristatus* 4.1%. Low counts were recorded for the remaining anurans *H. arborea* (5.8%) and *P. lessonae* (4%). Road-kill was lower than expected (percentage of road-kill as a proportion of live counts) in *R. lessonae* (5%), *T. marmoratus* (25%), *H. arborea* and *L. helveticus* (both 28%) but higher in *R. dalmatina* (120%) and *B. bufo* (123%). Larger body-size classes were the most frequent in most species. However, a skewness test, that evaluates the degree of overlap of two times the standard error of skewness with the skewness value (Tabachnick & Fidell, 1996), indicated that this was significant towards large individuals only in *H. arborea* and towards smaller animals in *R. dalmatina* (Table 1).

Monthly mortality

Figure 3 shows cumulative mortalities from the six-year study period. Amphibian mortalities were found throughout the year (mean of months from all data=62.4±56.8; min.=7, max.=212) but were irregular and highest in autumn and early winter (χ^2=618.8, *p*<0.0001). *B. bufo* road-kill was most frequent during October and November (57% of total casualties) including large females (76.6% versus rest of the year 57.5%, *z*=2.52, *p*=0.0056); adult casualties decreased during this period (40.4% versus rest of the year 53.7%, *z*=2.19, *p*=0.01). Most *R. dalmatina* casualties were found between July and November of which 44.6% were large adults, 26.2% subadults and 29.2% juveniles. Large adult casualties increased to 77% between November and June (*z*=4.04, *p*<0.00001) and were the only size class found between January and May.

Synchronous patterns of monthly road casualties were found between the urodeles; *T. marmoratus* and *T. cristatus* (*r*=0.81, *p*=0.001) and both species with *L. helveticus* (*r*=0.9, *p*<0.0001 and *r*=0.79, *p*=0.002) suggesting similar patterns of movement and road activity. Monthly road-kill showed no correlation with monthly sighting/capture frequency in any species; Pearson correlation coefficients from 0.01 (*P. lessonae*) to 0.31 (*L. helveticus*), *p*-values from 0.32 to 0.96.

Road and habitat specific mortality

Traffic intensity after sunset on the surveyed roads was as expected lower than during daytime (Meek, 2009). Levels varied between 10–21.1% (*mean*=14.2±3.8%) of daytime traffic and ranged from 0.8hr⁻¹ (Rue de Bourneau) to 17.2hr⁻¹ (D949). Road-kill ranged from 161 (Rue de Bourneau) to 9.2 per km (St Denis Du Payré to Chasnais). Mean road-kill per kilometre on 6 roads was 38.4±23.4 but the general pattern between roads was irregular (χ^2=85.3, df=5, *p*<0.001) as it also was within species - for instance *B. bufo* (χ^2=102.9, df=5, *p*<0.001) and *R. dalmatina* (χ^2=46.2, df=5, *p*<0.001). The highest *B. bufo* casualties were found on high traffic D949 but *R. dalmatina* and *L. helveticus* mortalities were prominent on the low volume traffic Rue de Bourneau that bisects urban and wooded/wetland areas (Fig. 1 & Table 2). Road-kill levels could not be significantly related to road traffic volume in a regression analysis of either total road-

Table 1. Size parameters of road-killed amphibians.

Species	Mean s.v. length (mm)	SD	Range	Skewness ± SE	*n*
B. bufo	71.6	24.9	14–121	0.13±0.14	294
R. dalmatina	48.3	13.5	18–87	0.35±0.17	191
P. lessonae	65.8	14.4	32–88	-0.12±0.44	30
H. arborea	39.4	5.1	26–50	-0.75±0.36	44
L. helveticus	31.9	5.8	13–44	-0.42±0.22	122
T. marmoratus	57.8	11.5	35–78	-0.36±0.35	47
T. cristatus	59.2	10.3	39–72	-0.78±0.43	31

Table 2. Number of road-kills per kilometre over a six year period. Road-kill was highest on the D44 and Rue de Bourneau. Rue de Bourneau and D127 bisect mostly wooded and/or wetland areas, D949 mostly monocultures and urban areas. For road locations see Fig. 1.

	D949	D60	D44	D127	Rue de Bourneau	Rue de Moulins	Mean±SD
Night-time traffic (mean hr^{-1})	17.2	10.0	8.4	2.3	0.8	1.1	6.6
B. bufo	80.0	12.6	42.5	7.5	15	5.5	27.2±26.6
R. dalmatina	2.5	11.2	15.0	24.2	55	2.5	18.4±18.0
H. arborea	0	2.2	17.5	2.5	7	0	4.9±6.2
P. lessonae	2.5	2.0	4.2	2.2	3	0.3	2.4±1.2
T. marmoratus	0	0.5	18.3	5.8	12	0.2	6.1±6.9
T. cristatus	0	2.3	5.0	2.5	4	0.5	2.4±1.8
L. helveticus	0	2.8	8.3	23.3	65	0.2	16.6±23.1
Total per km	85	33.6	110.8	68.0	161	9.2	

kill for each species (*regression coefficient*=0.05, *t*=0.11, *p*=0.91) or total road-kill for all species on each road versus mean road traffic volume for each road (*regression coefficient*=0.78 *t*=1.53, *p*=0.20).

In general, significantly greater than expected mortalities were recorded on low traffic roads bordered by woodland and/or wetlands in *R. dalmatina* and *L. helveticus* and to a lesser extent in *B. bufo* but less than expected in *P. lessonae* and *T marmoratus*. Road-kill in urban areas was significantly greater than expected in *T. marmoratus*, *P. lessonae* and *B. bufo*. However, road-kill next to monocultures was found to be significantly less than expected in all species tested. Observed cells of <5 road-kill in *T. cristatus* and *H. arborea* rendered analysis inappropriate with the χ² test. Table 3 summarizes the results including percent deviations (±) of road-kill from the expected frequencies.

Road-kill and precipitation.
Most road-kill was found after periods of either light or heavy rain (84.4% of total sample). Mortalities after dry weather were more frequent in anurans (mean=20.5% of 4 species) than urodeles (mean=2.7% of 3 species) especially in *R. dalmatina* (35.1%) and *P. lessonae* (20.0%). All dry weather road-kill in *P. lessonae* was between July and October, which are the months when many aquatic habitats desiccate. Figure 4 summarises the data.

Mortality and migratory distances.
Road-kill risk (R_{kill}) was assessed by a regression of total road-kill for each species against corresponding migratory distances M_d for the seven species and gave

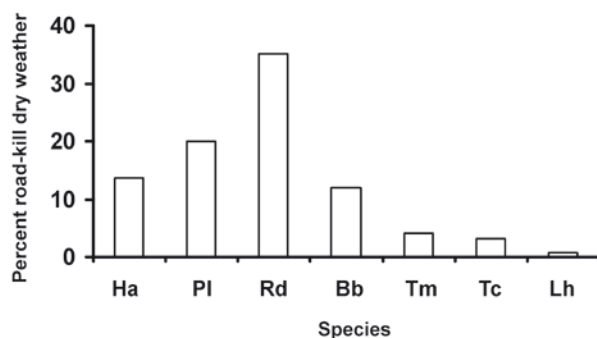$$R_{kill}=0.092\pm0.02M_d+32.9, \ r^2=0.87, \ n=7 \quad (1)$$

Fig. 4. Inter-specific differences in road-kill during dry weather expressed as percentages of the totals for each species. Species abbreviations are given in Fig. 2.
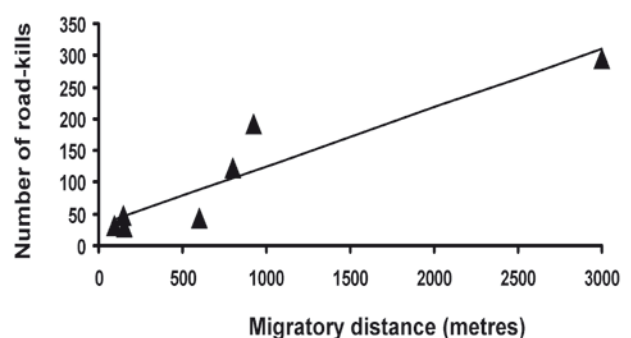

Fig. 5. Migratory distances plotted against number of road-kills. The lines running through the data are derived from equation (1). See text for details.

Table 3. Expected versus actual road-kill on roads bisecting habitat types. The results are derived from χ^2 tests of percentage deviations from expected road-kill based on a null model of random distribution (see text). The χ^2 values are corrected for continuity. Negative numbers indicate less than expected in terms of percent deviation. All tests are at df=2.

	χ^2	p	Urban (%)	Woodland/Wetland (%)	Monocultures (%)
B. bufo	27.7	<0.0001	39.0	14.3	-35.2
R. dalmatina	29.9	<0.0001	-12.4	51.3	-37.1
P. lessonae	12.8	0.0016	109.2	-5.57	-59.1
L. helveticus	63.7	<0.0001	-41.7	97.4	-59.8
T. marmoratus	41.3	<0.0001	167.1	-45.7	-58.3

with the coefficient significantly different from 0 (t=5.6, p=0.002); extent of migratory distances increases road-kill risk. The regression line is shown in Fig. 5. Removal of the arbitrary *P. lessonae* data point (see Methods) made little difference to the regression coefficient and gave 0.089±0.02 with t=4.9, p=0.008 (this now becomes n=6) against the 0 hypothetical coefficient. In Fig. 5 the data point for *R. dalmatina* lies outside the 95% confidence interval indicating higher than expected road-kill in relation to migratory distance. This may involve additional mortalities sustained during summer foraging and supports the notion that vagile species are at higher risk outside the migratory period.

Estimates of *B. bufo* and *R. dalmatina* biomass losses to road-kill.

If the size or biomass distribution of road-kill is affecting all sections of the population equally, then it should be in agreement with the biomass distributions of live samples. To test this assumption, biomass frequencies of very large *B. bufo* were arbitrarily set at s.v. length ≥80mm and large *R. dalmatina* at s.v. length ≥65mm. Road-kill biomasses in these large animals was calculated from the equations described in `Methods` and compared to biomass proportions in live animals (*B. bufo* n=43; *R. dalmatina* n=41). The results showed a significantly smaller number of large *R. dalmatina* were killed than expected (road-kill 51.1% versus live 68.6%, z=1.8, p=0.035) but significantly more large *B. bufo* (road-kill 77.7% versus live 53.2%, z=3.165, p<0.0008). Biomass proportion of large road-killed *R. dalmatina* increased during the migratory period (October through January, 60.1%) to closer agreement with the live sample (z=0.663, p=0.225). This result indicates road-kill had a greater impact on large female *B. bufo* biomass than other sections of the population.

DISCUSSION

Inter-specific differences in amphibian road-kill were largely associated with extent of movement, habitat type and precipitation. Roads that bisect wetlands/woodlands had high *R. dalmatina* and *L. helveticus* mortalities but on those bordering monocultures road-kill was less than expected, perhaps indicating the effect of agriculture on amphibian numbers. The expected temporal aspect of road-kill was confirmed with lower casualties during

spring and summer, when most species were either in aquatic (breeding) or terrestrial phases. The absence of a significant correlation with road traffic volume appears to be due to habitat related mortalities; for instance the highest overall road-kill was found on the road with the lowest traffic volume (Table 2). Extensive autumn amphibian migration in Vendée and subsequent high road-kill is unusual in Europe, which usually occurs in spring (e.g. Beebee & Griffiths, 2000; Puky, 2006).

As predicted vagile species, for example, *B. bufo* and *R. dalmatina,* were over represented and the sedentary *P. lessonae* under represented in the sample. This supports the notion of movement outside normal home ranges as the period of elevated risk (Carr & Fahrig, 2001; Puky, 2006; Sillero, 2008; Hartel et al., 2009). The low *P. lessonae* road-kill appears to be due to a sedentary lifestyle (Carr & Fahrig, 2001) and aestivation when ponds dry out. During long spells of dry weather large numbers were found in drainage tunnels and ditches that pass under roads (personal observation) and it may be relevant that the related *P. esculentus* entered tunnels constructed as amphibian underpasses more readily than *R. dalmatina* (Lesbarrères et al., 2004). Low road-kill in *H. arborea* is perhaps unexpected given that in some years this species is locally abundant and although it must migrate to ponds for breeding, short distance movement has been recorded (Meek, 2011), which might suggest low road-crossing frequency.

High levels of road-kill and hence biomass losses, particularly due to mortalities in large female *B. bufo* and *R. dalmatina* over a long time period, could substantially reduce the reproductive output of a population. An approximate estimate of road-kill effect has been calculated for adult *R. dalmatina* in the wooded/wetland area bisected by D127 and Rue de Bourneau using spawn clumps to estimate adult population size (Hill et al., 2005). During 2010, 32 large adults were road-killed on the short road segments whilst in adjacent wetland 85 spawn clumps were counted during the reproductive season (February 2011). An assumed male biased sex ratio of 3:1 (data from live captures and Racca, 2004) gives around 250 breeding adults, which approximates to road-kill taking about 11% of the adult population during 2010. The calculations do not take into account, among other things, mortality during winter dormancy or natural mortality leading up to the breeding period but

they apply to roads with very low volume traffic (Table 2) and illustrate the potential impact of traffic growth.

Road-kill must be less critical for those species that suffer high natural mortalities. For example, road-kill was estimated to be only around 3–4% of overall mortalities compared with annual mortalities of around 60% in migrating *B. bufo* in the UK (Gittins, 1983) - although see Langton (2002) for an alternative view. Species that are able to avoid roads are less likely to suffer negative effects than those that do not (Jaeger and Fahrig, 2001) but many amphibians must access summer home ranges and breeding areas and cross roads in the process. Furthermore, if roads render habitats on the opposite side of the road inaccessible this may reduce gene flow between populations (e.g. Lesbarrères et al., 2003; Shepard, et al., 2008). In France, Lesbarrères et al., (2006) found a heterozygosity deficiency in *R. dalmatina* populations living next to roads.

The results are in good agreement with road-kill data from other areas of Europe (e.g. Blab, 1986; Sinsch, 1988; Cooke and Sparks, 2004; Puky, 2006; Orlowski et al., 2008; Hartel et al., 2009; Elzanowski et al., 2009) including France (Lodé, 2000) but give only limited insight into effects on population levels. In part this is due to population fluctuations masking road-kill effects (e.g. Pechmann, et al., 1991) and difficulties in detecting road-kill due to rapid carcass degradation, which influence counts. Road-kill finds are probably biased towards amphibians with more durable skins. For instance, a small sample of carcasses tracked for road persistence indicated *B. bufo* (mean=17.0±13.8, range 1–36 days) carcasses were intact and visible for longer than other anurans (mean=5.6±4.9, range 1–16 days; t=2.6, p=0.02, df=13). Additionally, during the dry summer mummified carcasses of *R. dalmatina* remained on roads for longer than during wetter autumn weather (t=2.49, p=0.03, df=10). Extrapolation of the present road-kill data using proposed correction factors (Hels & Buchwald, 2001; Elzanowski et al., 2009) would suggest road-kill risk is actually highest in urodeles, particularly *L. helveticus*, due to rapid carcass degradation.

Road-kill risk is predictable since it is mostly associated with migration, which commences during the first heavy rains in October when median air temperatures ranged from 8–11.5 °C. Mitigation efforts during this period may require relatively inconvenient measures such as temporary road closures on very low volume roads with easy access to alternative routes. The Rue de Bourneau in the study locality is a good example; it has high habitat-associated amphibian mortality but very low traffic volume even during daytime (Meek, 2009) and there are alternative roads in the vicinity. Nevertheless, the economic importance of certain roads makes mitigation a more complex problem and a series of permanent measures may be needed. In France, mitigation measures, including those for amphibians, have been initiated for major new roads since 1984 (Mougey, 1996). Once high-risk areas and crossing points have been identified underpasses are a useful solution. In some instances they could have limitations, for example in species that are reluctant to enter tunnels (Lesbarrères et al., 2004) and

predators could learn to increase search opportunities at the point of entry or exit (e.g. polecats *Mustela putorius*; Lodé, 1996). However, management policies to protect migrating amphibians have been developed (Banks et al., 1994) and materials designed and tested for practical solutions are now available (Langton, 2002).

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