Long-term observations on the number of roadkilled *Zamenis longissimus* (LAURENTI, 1768) in a hilly area of Central Italy

Patterns of snake roadkills have been studied in different geographic and environmental contexts (e.g., ROSEN & LOWE 1994; BONNET et al. 1999; MEEK 2009; RAHMAN et al. 2013) because they can uncover issues in demography, ecology and population biology of such elusive vertebrates (ROW et al. 2007), as well as because high rates of roadkilled individuals may represent an effective threat to populations (CIESIOLKIEWICZ et al. 2006) and species (SHEPARD et al. 2008). In particular, long-term surveys of snake roadkills in one and the same study area may give insights into population trends as well as on the variables correlated with death-on-road of these reptiles (CAPULA et al. 2014). In this paper, the authors analyze data from 25 years of survey (between January 1987 and December 2011) of roadkills of the Aesculapian Snake, *Zamenis longissimus* (LAURENTI, 1768), in a hilly area of Mediterranean Central Italy (Tolfa Mountains, Latium), with emphasis on the effects of rainfall, ambient temperature, month and habitat type surrounding the roads, on the number of observed roadkills.

All roadkilled snakes encountered along the roads surrounding and interconnecting the villages of Canale Monterano, Manziana, Oriolo Romano, Monte Virginio, Vejano and Barbarano (altitudes ranging from 250 to 450 m a.s.l.; 40 km of road network surveyed) were recorded. Observed roadkills were removed in order to avoid multiple counts of the same individual and, where possible, sexed by examining the condition/shape of the cloacal area. Individuals exhibiting the subadult coloration were considered juveniles. During the investigations, individuals were neither collected nor preserved. As all data were collected opportunistically, there was no standardized survey effort: while some sections of the main roads were patrolled by car on 8-10 days per month, resulting in a minimum of 16-24 hours of survey per month, others were patrolled only once a month for about one hour or less. Patrolling was done during the routine activities of the authors, so the overall sampling effort was similar across years. However, since survey time data is not available, it cannot be excluded that the results are biased due to non-standardized survey effort. The following climatic data were obtained: (i) mean annual air temperature for the locality 'Canale Monterano-Viterbo' available from < http:// www.meteo.it >), (ii) yearly number of rainy days (rainy days, 1-10 mm) and (iii) yearly number of storm days (> 10 mm of rain) for the locality 'Bracciano' (available from < http://www.idrografico.roma.it >). Mean monthly temperatures were not considered for further analyses although available in the data matrix, because they were significantly positively correlated with the corresponding mean annual temperatures (authors' unpublished data). Over the study period there was a significant increase in the mean annual temperature (r = 0.646, p < 0.001), whereas rainfall remained unchanged (rainy days: r = 0.093, p = 0.658, storm days: r = -0.139, p = 0.518). Each roadkilled snake was assigned to the type of habitat to which it was closest at the time of death. The following habitat types were distinguished: (i) urban (includes any condition with evident 'cement structures' outside the road); (ii) farms and plantations; (iii) grassland; (iv) bushes; (v) woodland; (vi) marshlands and water bodies. Non-normally distributed variables were $\log_{10}$-transformed prior to statistical analyses. Pearson's correlation coefficient was used to analyze the relationships between number of roadkills per year and the three climatic variables presented above. Observed-versus-expected χ^2 test was used to analyze (i) the differences in the frequency of roadkilled males, females and juveniles, (ii) the monthly differences in the number of recorded roadkills, and (iii) the frequency differences among habitat types in terms of number of roadkilled snakes. Alpha was set at 0.05, and analyses were performed using the statistical software PAST (v. 3.0).

Overall, a total of 164 roadkills were observed during the 25 years of study (mean = 6.96 ± 5.8; median = 5). The num-

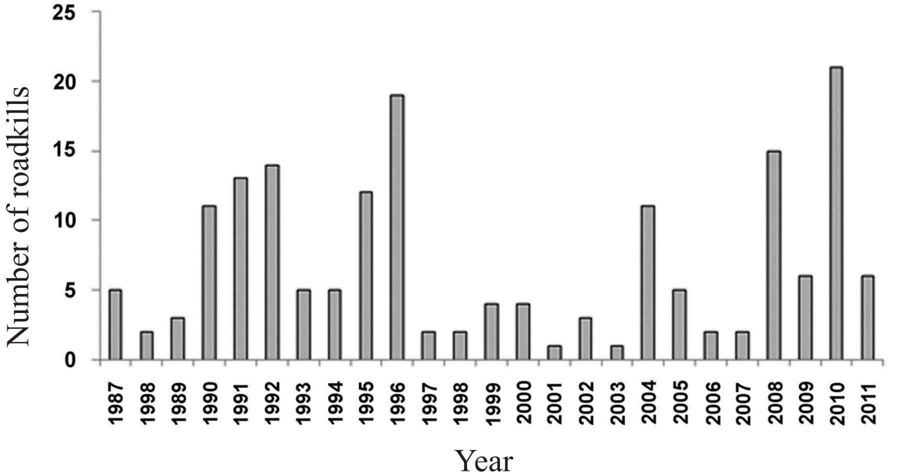

Fig. 1: Number of roadkills of *Zamenis longissimus* (LAURENTI, 1768), observed in the study area (Tolfa Mountains, Latium, Central Italy) in the years 1987 through 2011.

ber of roadkills varied remarkably among years (range: 1-21), with most individuals observed in 1996, 2008 and 2010. Although there was no obvious pattern throughout the years, there were periods with high numbers of roadkills (1990-1992, 1995-1996) alternating with periods of low numbers of roadkills (1997-2003, 2005-2007) (Fig. 1).

Sex was determined in 145 roadkills: adult males (N = 91) statistically dominated over adult females (N = 43) and juve-

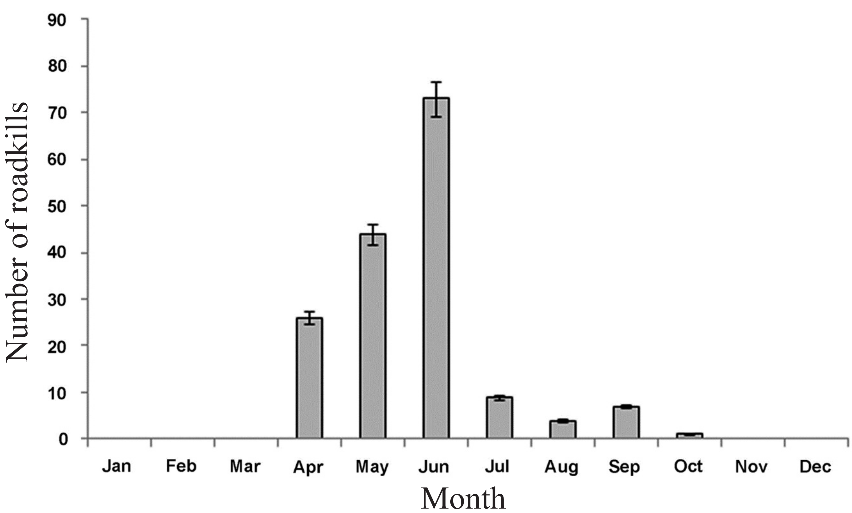

Fig. 2: Numbers of roadkilled *Zamenis longissimus* (LAURENTI, 1768) in the study area (Tolfa Mountains, Latium, Central Italy). The columns represent the sums of roadkills in the years 1987-2011 arranged by month. Vertical bars on top of the columns indicate standard errors.

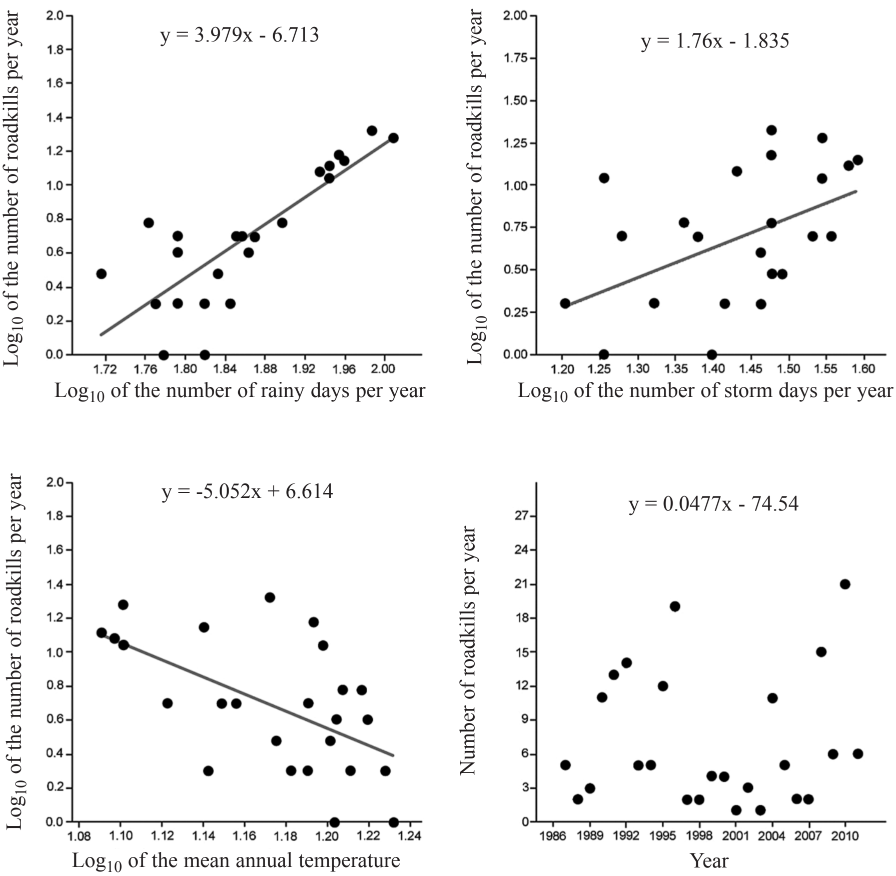

Fig. 3: Relationships between the yearly number of roadkilled *Zamenis longissimus* (LAURENTI, 1768) in the study area (Tolfa Mountains, Latium, Central Italy) and three climatic variables plus the timespan. The regression line equations are given above each diagram. For further statistical information, see text.

niles (N = 11) ($\chi^2 = 67.1$, $df = 2$, $P < 0.0001$). Pooling together all data coming from the whole timespan of study, the monthly distribution of roadkill sightings was significantly uneven ($\chi^2 = 148.3$, $df = 11$, $P < 0.0001$) with peaks in May and June (Fig. 2). Unfortunately, this analysis could not be done by year because in most years (56 %; N = 25) the number of recorded roadkills was too low (≤5 per year). Nonetheless, the variation in the standard error for groups of five years was moderate (authors' unpublished statistics), suggesting that the monthly patterns were stable all throughout the timespan of the study.

The $\log_{10}$ of the number of roadkills per year was positively correlated to both the $\log_{10}$ of the number of rainy days per year ($r^2 = 0.686$, N = 25, $P < 0.0001$) and the $\log_{10}$ of the number of storm days per year ($r^2 = 0.233$, N = 25, $P < 0.05$; Fig. 3). The $\log_{10}$ of the number of roadkills per year was negatively correlated to the $\log_{10}$ of the mean annual temperature ($r^2 = 0.326$, N = 25, $P < 0.01$; Fig. 3), but this relationship was probably dependent also on the

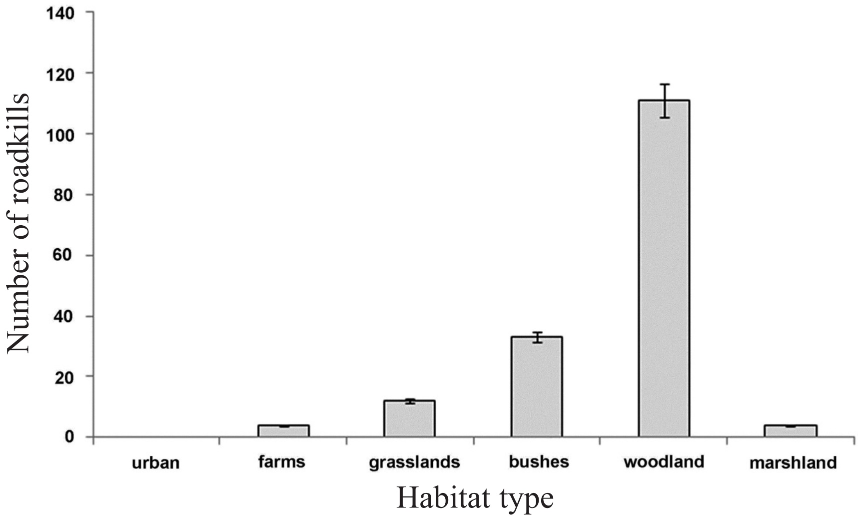

Fig. 4: Number of roadkilled *Zamenis longissimus* (LAURENTI, 1768) in the study area (Tolfa Mountains, Latium, Central Italy) by adjacent habitat type. Most roadkills were found in those sections of road that were surrounded by woodland and dense bushes. Vertical bars on top of the columns indicate standard errors.

significantly negative relationship between $\log_{10}$ of the number of rainy days per year and $\log_{10}$ of the mean annual temperature ($r^2 = 0.236$, N = 25, $P < 0.05$; Fig. 3). Indeed, despite there was a significant increase over the study period in the mean annual temperature ($r = 0.646$, $p < 0.001$; but not in rainfall regime; rainy days: $r = 0.093$, $p = 0.658$, storm days: $r = -0.139$, $p = 0.518$), there was no significant increase/decrease in the number of roadkills over the years ($r^2 = 0.0027$, N = 25, $P = 0.806$; Fig. 3). As a general pattern, roadkills were observed more frequently in segments of road that were surrounded by mixed oak forest (*Quercus* spp.) or dense bushes (e.g., *Rubus ulmifolius*, *Cytisus scoparius*, etc.) along the roadside ($\chi^2 = 119.2$, $df = 5$, $P < 0.0001$; Fig. 4).

Beginning the discussion it should be stressed that this study is correlational, and just provides evidence that the numbers of roadkills, understood as a measure of activity intensity and perhaps population size of the studied species along roads in the study area, have changed in parallel with rising temperatures and, particularly, oscillations in the rainfall regime (see also RUGIERO et al. 2013; CAPULA et al. 2014 for other case studies with Italian snakes). It is obvious that the length of stay or the way in which snakes utilize roads (e.g., motionless for thermoregulation or rapidly crossing the construction when moving within the habitat) can influence the roadkill numbers. Habitat modification such as increase or decrease in shrub density alongside the roads occurred during the study period of two decades might have altered snake activity (CAPULA et al. 2014).

In this study, the great majority of roadkills occurred in May and June (with moderate variation across years), which is the species' local period of mating (CAPULA et al. 1995; CAPULA & LUISELLI 1997). Since adult males formed the majority of roadkills, most cases of death on roads were likely to refer to adult males vagabonding in search of a female mate. The same pattern was found in Western France (BONNET et al. 1999) as well as in *Hierophis viridiflavus* (LACÉPÈDE, 1789) from Central Italy (CAPULA et al. 2014), whereas MEEK (2009) observed the highest mortality in juveniles after hatching (August to October) for *H. viridiflavus*, and in June for *Natrix natrix* (LINNAEUS, 1758) in Vendée, France.

There was an evident correlation between the presence of a roadkilled *Z. longissimus* and the adjacent habitat type, with the great majority of dead individuals being recorded in the surrounding of woodlands (*Quercus* spp.). This is attributable to (i) a well known preference of this species for woody habitats in Central Italy (e.g., LUISELLI & RUGIERO 1990; LUISELLI & CAPIZZI 1997; FILIPPI & LUISELLI 2006), and (ii) an ecotonal effect (edge effect) of basking benefits (MEEK 2009), with snakes using roads also to warm up more efficiently in an otherwise relatively shaded habitat as it is the woodland habitat.

It seems that considerable complications can hamper reliable predictions of the relative vulnerability of temperate snakes along roads (BONNET et al. 1999; MEEK 2009), and that site-specific characteristics (intensity of traffic by season and by daytime, temperature and humidity, habitat characteristics, nearby roads) greatly contribute to the variability of the observed patterns. Nonetheless, the present data is noteworthy in particular when compared to those collected over the same years, on roadkilled *H. viridiflavus* in the same study area (CAPULA et al. 2014). Despite (i) the numbers of yearly roadkilled individuals were comparable in the two species, and (ii) there was no apparent declining/increasing trend in the yearly number of roadkills over the years (thus suggesting no change in overall population sizes along the 25-years-long study period), the two species differed remarkably in terms of the observed roadkill temporal patterns. In *H. viridiflavus* the mean annual temperatures were positively correlated with the number of roadkills, whereas rainfall was not correlated (CAPULA et al. 2014). In *Z. longissimus* however, the rainfall-linked variables were positively correlated with the roadkill numbers in each year. Mean annual temperature was negatively correlated with the number of roadkills, but probably just because it was also negatively correlated to both rainfall variables considered in the analysis. After all, data suggests that increasing rainfall (= humidity of the air) caused a higher above-ground activity intensity of *Z. longissimus*, and especially of the adult males that were the main category exposed to death on the road. While earlier studies on sympatric populations of *Vipera aspis* (LINNAEUS, 1758) and *H. viridiflavus* suggested that global warming has generally resulted in shifts in the ecology and phenology of Mediterranean snakes (RUGIERO et al. 2013; CAPULA et al. 2014), the present study did not show any significant effect of ambient temperature rise on *Z. longissimus* roadkill frequency, whereas changes in the rainfall regime patterns could be responsible for the inter-annual variations in the above-ground activity intensity. One should, however, bear in mind that consistent changes in the rainfall regime may be caused by global warming. Heavy rainfall was also correlated with peaking snake roadkills also in tropical areas of Bangladesh (monsoon season; RAHMAN et al. 2013) and Nigeria (onset of the rainy season; AKANI et al. 2002), but this relationship was never highlighted in previous studies on temperate zone snakes.

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