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Long-term, climate change-related shifts in monthly patterns of roadkilled Mediterranean snakes (*Hierophis viridiflavus*)

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Ectothermic vertebrates depend on ambient temperatures for their activities. Thus, global warming is expected to influence several aspects of their ecology. Here, we use a >20 year (1990–2011) dataset on monthly numbers of roadkills in an area of central Italy in order to document whether the phenology of a Mediterranean population of Western whip snakes (*Hierophis viridiflavus*) has changed over time. Annual variation of roadkills was correlated to five climatic variables: (i) mean annual air temperature, (ii) mean February air temperature, (iii) mean July air temperature, (iv) yearly number of rainy days, and (v) number of rainstorm days. Increases in mean annual temperature were positively related to the number of roadkills at the early (February and March) and late (December) phases of above-ground activity, but were negatively related to the number of roadkills during summer (June and July). Intriguingly, we documented a shift in the annual mortality peak over the study period, possibly indicating temporal changes of the mating season due to global warming. Increasing mean air temperatures apparently caused an earlier onset of above-ground activity of snakes and delayed hibernation, but reduced the intensity of snake above-ground activity during the hottest and driest period of the year. Rainfall variables had no impact on snake activity.

Key words: global warming, phenology, roadkills, snakes

INTRODUCTION

There is a general consensus that climate change will result in increased air temperatures in most regions of the world together with increased frequencies of extreme weather events (Easterling et al., 2000), and living organisms are required to adapt to these changing conditions if they want to survive (Grabherr et al., 1994; Guisan & Zimmermann, 2000; Araujo et al., 2006). Reptiles are ectothermic vertebrates sometimes coupled with temperature-dependent sex determination (Janzen, 1994), and climate change is thus expected to directly affect their ecology and life-histories (e.g., Araujo et al., 2006). Therefore, long-term monitoring data on reptilian populations are needed to describe phenological and life-history changes due to climate change (Ujvari et al., 2011; Rugiero et al., 2012a, 2013a).

An indirect way to monitor population trends in snakes is the analysis of roadkilled specimens (Meek, 2009), and roadkill data are a reliable proxy of activity intensity for active and mobile species (Bonnet et al., 1999; Rahman et al., 2013). In temperate regions, peaks of snake roadkill mortality occur during the mating season in males (when they disperse for searching for a mate) and during the oviposition phase in females (when they disperse in search of a good oviposition site, Bonnet et al., 1999). Another common cause of snake roadkills may be basking activity related to "in-road immobilisation behaviour" (Volkl & Thiesmeier, 2002; Andrews et al., 2005).

In this paper, we use monthly numbers of roadkills observed in the same study area for over twenty years in order to document whether the phenology of a Mediterranean population of snakes has changed over time in relation to climate change (as already observed in another temperate snake species, Rugiero et al., 2013a). As a study model, we used the Western whip snake (*Hierophis viridiflavus*: Colubridae), the most common snake species in central Italy (Vanni & Zuffi, 2011). Roadkill patterns for this species were previously documented for populations from western France (Bonnet et al., 1999) over a shorter periods of time.

MATERIALS AND METHODS

Hierophis viridiflavus is a medium-sized (up to 150 cm long), oviparous, colubrid snake which feeds mainly upon lizards, small rodents and small birds (Rugiero & Luiselli, 1995; Vanni & Zuffi, 2011; Lelièvre et al., 2012). In the

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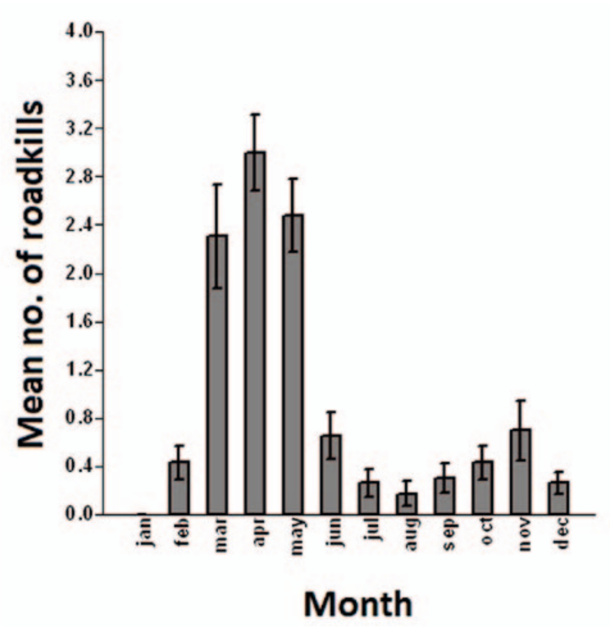


Fig. 1. Mean (and standard error) monthly distribution of the number of roadkilled *Hierophis viridiflavus*.

study area, this snake is mainly active above-ground from March to November, and most of the females reproduce every year (Filippi et al., 2007; Luiselli et al., 2011a). The range of optimum body temperatures (T_{set}) is 27.5–31°C (Lelièvre et al., 2010), which is at the upper limit of all Mediterranean snakes studied to date (Vanni & Zuffi, 2011). This species also shows considerable movements and home ranges (Rugiero et al. 2012b, 2013b), and is one of the least threatened Italian snakes (Filippi & Luiselli, 2000).

The study was carried out between January 1990 and December 2011 in a hilly area in northern Latium, central Italy, as part of concurrent long-term studies on snake ecology (e.g., Luiselli et al., 2011b; Rugiero et al., 2012a, 2013a). All roadkilled snakes encountered along the roads surrounding and interconnecting the villages of Bracciano, Canale Monterano, Manziana, Oriolo Romano, Vejano and Barbarano (altitudes ranging from 250 to 450 m a.s.l.) were recorded. Observed roadkills were removed in order to avoid multiple counts of the same individual. During our investigation no individual was collected and preserved.

The data were collected opportunistically without standardised survey efforts. Some sections of the main roads were patrolled by car for 8–10 days per month, resulting in a minimum of 16–24 hours of surveying per month and year. In total, approximately 40 km of roads were surveyed. Because the sampling regime was similar across years, we argue that the data are sufficiently unbiased for the drawn inferences.

The following climatic data were obtained from Alessandrini et al. (2008, see also Appendix 1): (i) mean annual air temperature (Av. T. year), (ii) mean February air temperature (Av. T. Feb.), (iii) mean July air temperature (Av. T. Jul.), (iv) yearly number of rainy days (Rainy days, 1–10 mm) and (v) number of rainstorm days (>10 mm of rain). There was a significant increase over the study period in Av. T. year (r=0.646, p<0.001) and Av. T. Jul. (r=0.471, p<0.05), whereas rainfall remained unchanged (rainy days: r=0.093, p=0.658, rainstorm days: r=-0.139, p=0.518; Appendix 1). However, Av. T. year and Av. T. Jul. were significantly positively correlated with each other (Table 1), and we therefore used only Av. T. year for statistical analyses. Alpha was set at 0.05, and analyses were performed using the statistical software PAST (v. 2.17) and SPSS (v. 11.0).

RESULTS

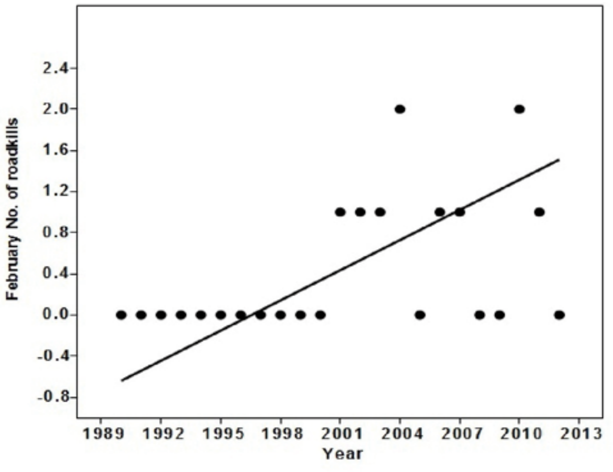
The number of roadkills per year ranged between 6 and 17, with no significant trend over the study period (r=0.013, p=0.953). The mean number of roadkills varied significantly across months (Kruskal Wallis ANOVA: H (χ^2)=96.7, p<0.001), with the highest number of roadkills occurring between March and May (Fig. 1). Excluding January from analyses (no roadkill encountered), the number of roadkills increased over the study period in February (r=0.486, p=0.019), March (r=0.656, p<0.001) and December (r=0.567, p=0.005), decreased in May (r=-0.548, p=0.007) and June (r=-0.545, p=0.007, Fig. 2), and did not change in April (r=-0.076, p=0.732), July (r=-0.276, p=0.209), August (r=-0.341, p=0.116), September (r=-0.299, p=0.164), October (r=-0.263, p=0.225) and November (r=0.158, p=0.470).

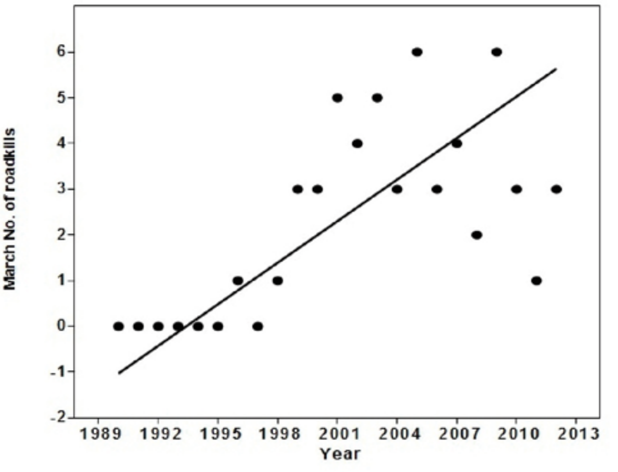
The number of yearly roadkills was significantly positively correlated with Av. T. year for February (r=0.441, p=0.040), March (r=0.774, p<0.001) and December (r=0.454, p=0.039, Fig. 3). A significant negative correlation was found between mean Av. T. year and number of roadkills in May (r=-0.618, p=0.003) and June (r=-0.547, p=0.010, Fig. 3). However, mean temperature in February was uncorrelated with the number of roadkills observed in that month (r=0.285, p=0.199).

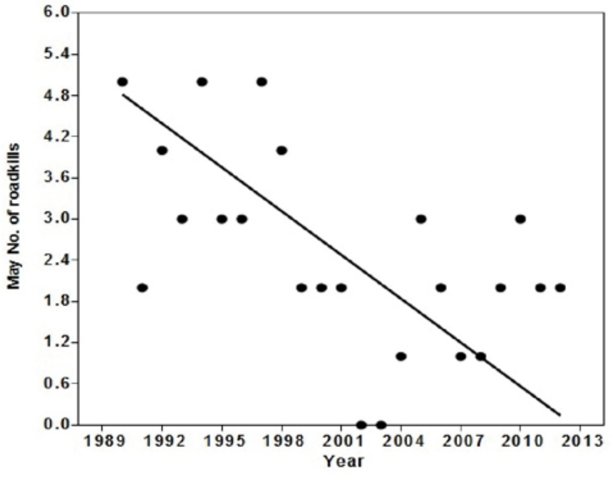
Yearly number of rainy days was not correlated with the number of roadkills observed in those months for which a long-term trend was observed (February: r=-0.06, p=0.979; March: r=-0.127, p=0.573; May: r=0.119, p=0.629; June: r=0.135, p=0.558; December: r=-0.269, p=0.238).

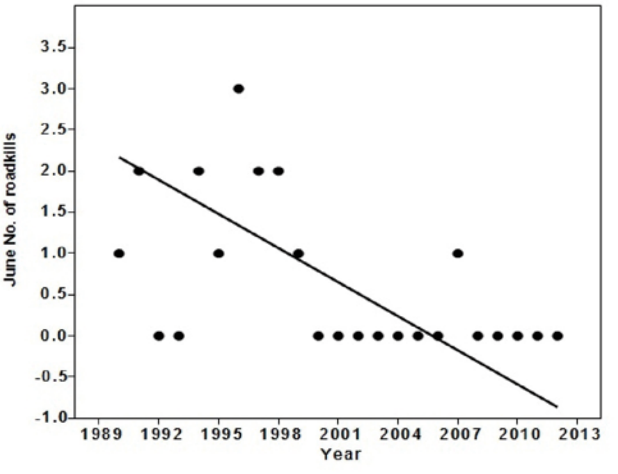
Table 1. Correlation analyses (Pearson's correlation coefficient on log-transformed data) between climatic variables used in this study. Significant correlations are shown in *italic*. T=temperature.

	Average T. Febuary	Av.erage T. July	Rainy days
Average T. year	*r=0.436 p=0.029*	*r=0.788, p<0.001*	*r=-0.486, p=0.0138*
Average T. February	******	r=0.239, p=0.251	r=-0.388, p=0.552
Average T. July		******	r=-0.297, p=0.149









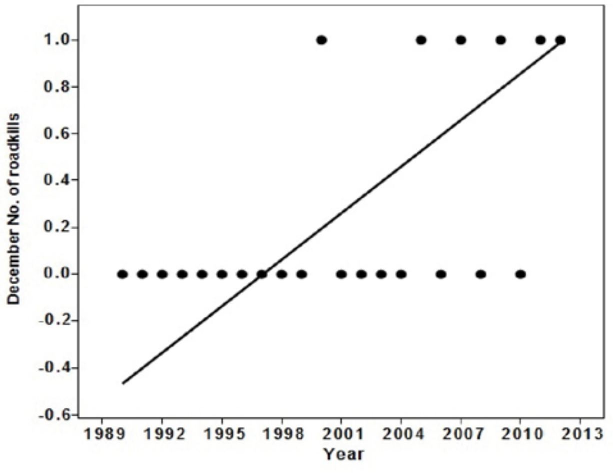
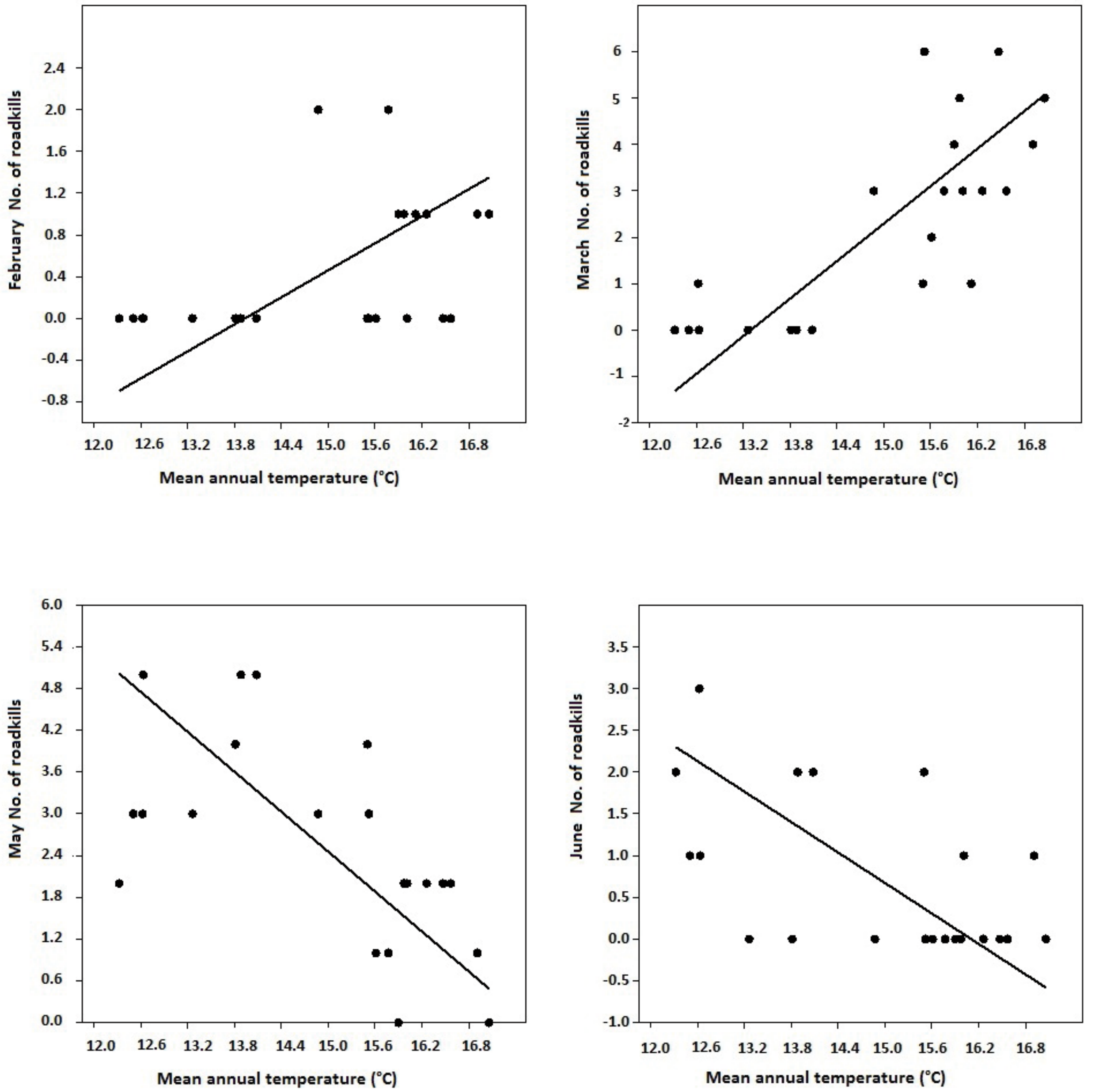


Fig. 2. Number of roadkilled *Hierophis viridiflavus* found per month over the study period. See text for details on statistical tests. Only statistically significant relationships are presented.

DISCUSSION

Although the results of this study are correlational, they provide evidence that the activity patterns of *H. viridiflavus* have changed in parallel with rising temperatures (see also Rugiero et al., 2013a for another case study with snakes). However, the use of roads by snakes can influence roadkill numbers (e.g., PACA, 2011), and habitat modification in the periphery of roads over the two decades of the study period might have altered snake activity. If movements are constrained by habitat loss, snake activity can be directed to habitats that attract them (roads as an optimal habitat for basking) or can result in dispersal to new habitats.

The uneven distribution of the monthly number of roadkills mirrors the seasonal activity patterns already documented for *H. viridiflavus* at the study area, with peaks in the number of roadkills synchronising well with the mating period occurring in April and May (Vanni & Zuffi, 2011). This is also consistent with evidence from western France, where road mortality peaks occur during the mating period and are biased towards males which move further and hence are more likely to cross roads (Bonnet et al., 1999). Our documented shift in the annual mortality peak therefore suggests that the mating period might have changed in parallel with rising temperatures (Rugiero et al., 2013a). The evidence that the number of roadkills in May and June decreased over the years suggests that these months no longer correspond to a phase of intense above-ground activity. After the mating period, snake activity tends to focus on foraging (e.g., Lelièvre et al., 2012), which might be less intense at higher (and potentially stressful) ambient temperatures (Rugiero et al., 2013a). Increased temperatures may however also cause individuals to be more active at earlier hours, reducing their risk to be roadkilled because of lower traffic intensity.



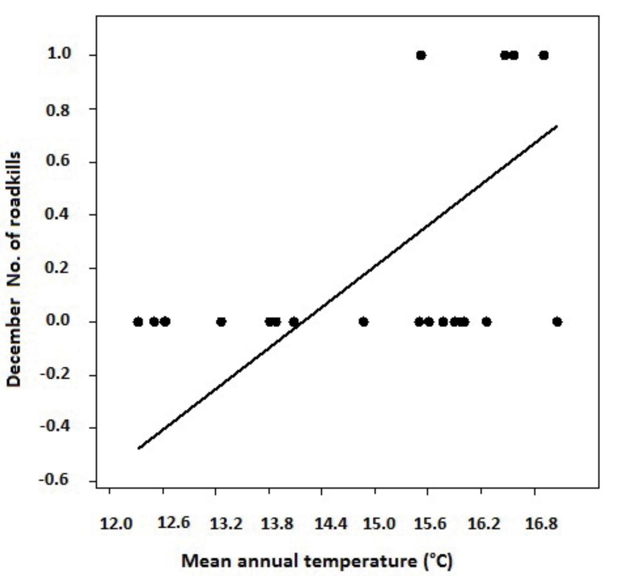


Fig. 3. Relationships between climatic variables and mean number of roadkilled *Hierophis viridiflavus* observed yearly in each month. Only statistically significant relationships are presented.

Mean annual temperatures influenced the number of roadkills but rainfall regime did not. More specifically, increases in mean annual temperatures positively influenced the number of roadkills at the early phase of the snake above-ground activity (February and March) and at its latest phase (December), whereas they negatively influenced the number of roadkills during summer (June and July). This suggests that increasing air temperatures caused an earlier onset of above-ground activity and a delayed hibernation, reducing activity during the hottest and driest period of the year. Overall, these data are in agreement with those on another species (*Vipera aspis*) in the same study area (Rugiero et al., 2013a). Both studies together suggest that global warming has generally resulted in shifts in the ecology and phenology of Mediterranean snakes. However, as yet we lack evidence that the intervening phenology shifts are affecting population sizes of *H. viridiflavus;* an increased time spent above-ground could for example translate into having more time to feed (see also Rugiero et al. 2013a for *V. aspis*). Quantifying the effects of global warming on Mediterranean snakes requires further studies from additional localities.

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APPENDIX

Appendix 1. Yearly variation of the climatic variables used.