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Regular research paper

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## HIGH JUVENILE MORTALITY OF GRASS SNAKES *NATRIX NATRIX* (L.) ON A SUBURBAN ROAD

**ABSTRACT:** Hundred-ninety dead grass snakes *Natrix natrix* (L. 1758) were collected over 10 months in two years on an 1800-meter stretch of a local road in the outskirts of Wrocław, a major city in SW Poland. The mortality rate reached a record high value of 204 snakes  $\text{km}^{-1} \text{ year}^{-1}$  (1.16 snakes  $\text{km}^{-1} \text{ day}^{-1}$ ). Two peaks of road mortality, one from the end of May through the beginning of June, and the other, at the beginning of August, contributed 80% of records. The majority (89%) of 110 measured specimens were juveniles with the total length below 30 cm, and around 30% of those collected in the spring and the summer were hatchlings with the total length up to 20 cm. This suggests a significant extension of the hatching period, which may be related to the local mild climate and/or climate warming. The road kill numbers correlated significantly with maximum daily temperatures through the cool (for May) to average (for June) spring of 2004 but not through the hot spring of 2003, which suggests that under average or cool weather conditions the mobility of grass snakes is limited by maximum daily temperatures. No significant correlation with daily rainfall could be established.

**KEY WORDS:** European grass snake, *Natrix natrix*, juvenile mortality, road mortality, Lower Silesia

### 1. INTRODUCTION

Grass snakes *Natrix natrix* (L. 1758) are widespread in Eurasia and prefer wet, diversified habitats with rich vegetation in the vicinity of water (Juszczyk 1987, Günther and Völkl 1996, Kabisch 1999, Beebee and Griffiths 2000, Sura and Zamachowski 2003). A clear decline of populations of this species during the last decade has been recorded in many areas of Europe (Kabisch 1999, Beebee and Griffiths 2000) including Poland (Sura and Zamachowski 2003). However, the specific causes of this phenomenon and the possible role of road mortality remain unclear.

Road mortality of snakes has been studied primarily in North America (Dodd *et al.* 1989, Bernardino and Dalrymple 1992, Rosen and Lowe 1994, Tucker 1995, Rudolph *et al.* 1998) and Asia (Gokula 1997), the only major European study being from Portugal (Brito and Álvares 2004). Most of the published Grass snake road mortality data are limited to the road-kill numbers, especially from snapshot censuses in the areas with high population densities (Bonnet *et al.* 1999). In Poland, Bartoszewicz (1997) recorded about 30 grass snakes  $\text{km}^{-1}$

year<sup>-1</sup> from an 11-km stretch of road crossing a variety habitats next to a nature reserve near the country's western border. Borczyk (2004) provided an estimate of 48.6 snakes km<sup>-1</sup> year<sup>-1</sup> from an unspecified stretch of road crossing a water-rich nature reserve in Lower Silesia, SW Poland, 60 km NE of Wrocław, one of Poland's major cities where the present study was conducted. Here, we provide data on the mortality of grass snakes on a suburban road crossing humid riverside habitats, with a particular attention to annual dynamics and age composition.

## 2. STUDY AREA

Surveyed for road kills was a 1800-meter stretch of a tarmac road in the outskirts of Wrocław (640 000 inhabitants; Lower Silesia, SW Poland; 17°02'E, 51°07'N). The surveyed road is located in the valley of Bystrzyca (Fig. 1), a small and mostly unregulated river. In the vicinity of the road there are eight ponds (0.16–1 ha) encircled with lush vegetation. The woodlands are dominated by broad-leaved trees and the forest floor is permanently covered by

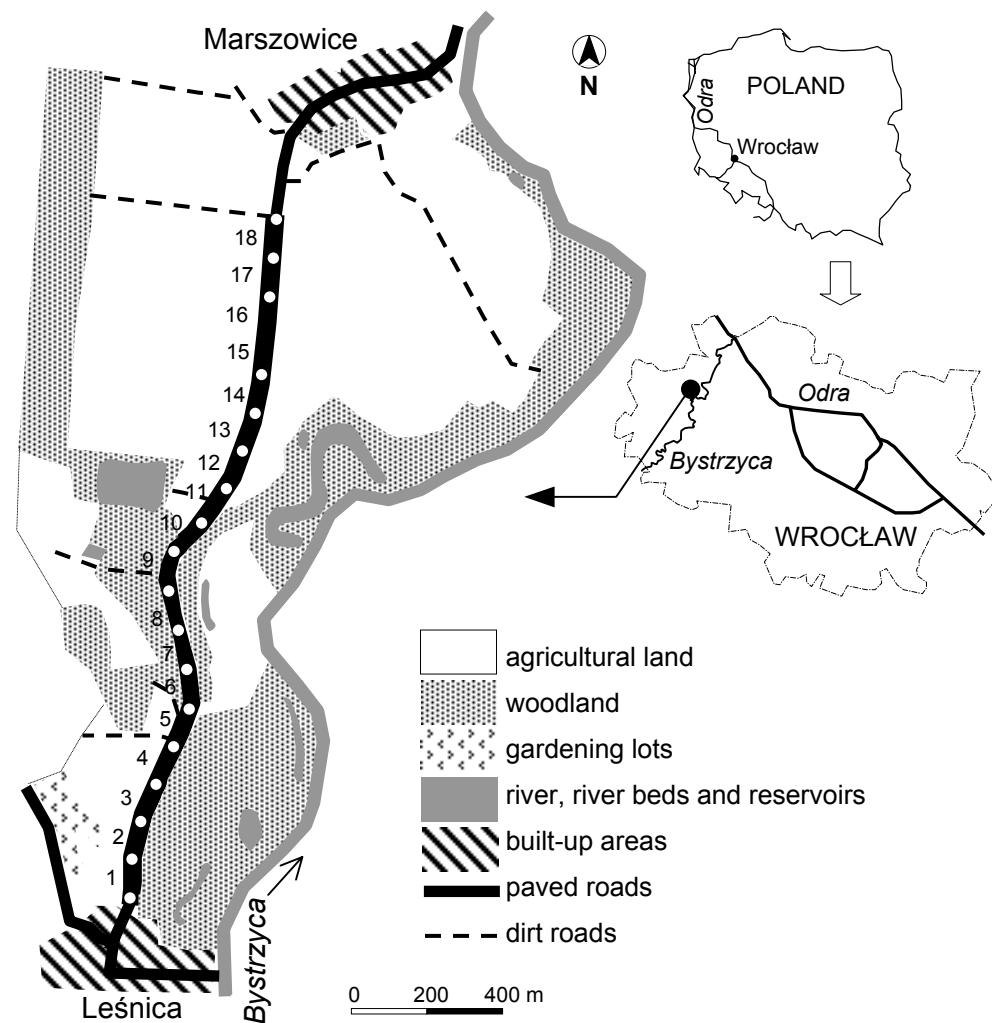


Fig. 1. Location of the 1800-meter stretch of a suburban road, where the mortality of Grass snakes *Natrix natrix* was recorded in years 2003–2004. Numbers mark 100-meter sections.

a thick layer of decaying leaves. Close to the river banks there are remnants of the natural riverside woodland (*Salici-Populetum*) with *Salix alba*, *S. fragilis*, *Populus nigra*, and *P. alba*. The surveyed road stretch also crosses abandoned cropland with a natural succession of plant communities. The road crown is 5 meter wide. The road shoulders are very narrow, about 0.5 m each and mostly covered by herbaceous vegetation. Based on our own census, the road is used by around 900 vehicles daily, mostly between 7 am and 6 pm. The surveyed road stretch was subdivided into eighteen 100-meter sections. Using detailed cartographic data, each of them was characterised in terms of the length of its borders to the adjacent woodland or field (for each border separately) and its distance to the closest body of water and to the bed of Bystrzyca river.

### 3. MATERIAL AND METHODS

The surveys were conducted from March 10 through November 20 in 2003, which includes the entire active seasons of grass snakes, and from March 16 through June 30 in 2004. Seventy-seven (49 in 2003 and 28 in 2004) of them were conducted within the 176-day road kill period from April 24 through October 16 (as determined for 2003). The road was surveyed by one of us every other day from March through May (because of the initial focus on amphibians) and 1–2 times a week in the remaining months. The surveys were performed between 6 am and 10 am by walking on one side of the road and returning on the other. All 190 road kills were removed from the road and transported to the Laboratory of Vertebrate Zoology, University of Wrocław, where the measurements of total body length (TL) could be taken for 110 individuals, and of snout-vent length (SVL) for 95 individuals, which is why we used TL in all further calculations. The measurement error was up to  $\pm 5$  mm because of frequent heavy damage and/or torpor mortis. Also because of damage, the majority of specimens could not be reliably sexed.

Based on extensive data for Poland (Juszczyk 1987), which are in a good agreement with data from northern Ger-

many (Günther and Völkl 1996), the total length ranges 17–19 cm (16.8 cm–20.6 cm) for hatchlings, 25–30 cm for yearlings, above 50 cm for mature males, and above 60 cm for mature females. Hatchlings, yearlings, and all stages in between are referred to as juveniles and those between yearlings and adults as subadults.

Temperature data were obtained from the Meteorological Station of the University of Wrocław, which is located in the SE part of the city, about 16 km from the study area. We used the mean ( $T_m$ ), maximum ( $T_{max}$ ), and minimum ( $T_{min}$ ) daily temperature, the minimum temperature 5 cm above the ground ( $T_{min} + 5$  cm), and the cumulative daily precipitation. We analysed the weather data from both the survey days and the preceding days because the surveys were conducted in the morning hours and thus probably included road kills of the previous day. We also obtained maximum temperature data for the last 10 years and mean temperature data for the last 40 years.

In the absence of normal distribution for the majority of variables (with  $P < 0.05$  in Kolmogorov-Smirnov test), all statistical analyses were performed with nonparametric methods. We used Wilcoxon test for comparisons of road kill numbers between the corresponding seasons of 2003 and 2004, Mann-Whitney test for comparisons of mean total length (TL) values for these two years, Kruskall-Wallis test for comparing total length (TL) between months, chi-square ( $\chi^2$ ) test for comparing the shares of age categories between months and for comparing differences between the real numbers of kills on each 100-meter road section and the mean (10.55, that is, 5.55% of 190), and Spearman rank correlation coefficient for calculating the correlation between road kill numbers and weather conditions.

The impact of habitat variables on the road-kill numbers was calculated using standard regression. Because of a strong correlation between the analysed independent variables ( $P < 0.05$ ), each of the regression models was calculated separately for every variable. Variables in the regression models showed a normal distribution.

#### 4. RESULTS

Altogether 190 dead grass snakes, 102 in 2003 and 88 in 2004, were found on the surveyed stretch of road. The earliest road kill was found on April 23 of 2004 and the latest on October 16 of 2003. For the 77 survey days, 46 (28 in 2003 and 18 in 2004) yielded at least one dead snake and the mean mortality was 1.38 snakes  $\text{km}^{-1} \text{ day}^{-1}$  which amounts to around 243 snakes  $\text{km}^{-1} \text{ year}^{-1}$  assuming a 176-day season (based on year 2003). However, this may be an overestimate since the 2004 surveys ended in June, making it safer to base the estimate on year 2003 alone, when the mortality rate was 1.16 snakes  $\text{km}^{-1} \text{ day}^{-1}$ , which amounts to a realistic estimate of 204 snakes  $\text{km}^{-1} \text{ year}^{-1}$ .

In 2003 there were two peaks of mortality (Fig. 2), in the late spring (May through the beginning of June) and late summer (August through mid-September), and the late spring

peak was even more pronounced in 2004. The great majority (80%) of all road kills occurred within these two peaks. The recorded seasonal pattern of mortality from April 20 through June 20 was similar for years 2003 and 2004 (Wilcoxon test,  $Z = 1.68$ ,  $P = 0.09$ , Fig. 2), which adds credibility to the generalizations based on the entire study period.

TL ranged from 15.0 to 78.5 cm. The mean ( $\pm \text{SD}$ ) TL values for years 2003 ( $24.1 \pm 11.6 \text{ cm}$ ,  $n = 56$ ) and 2004 ( $23.6 \pm 6.7 \text{ cm}$ ,  $n = 54$ ) were very close (Mann-Whitney test,  $U = 1329.5$ ,  $Z = -1.09$ ,  $P = 0.27$ ). There are no significant monthly differences in the mean TL, either for all months (Kruskall-Wallis test,  $H_4 = 1.29$ ,  $P = 0.86$ ,  $n = 109$ ) or for two months (in Mann-Whitney test range of  $P$ -value = 0.28–0.86), even though two longest snakes (78.5 and 74 cm) were found in May and June (Fig. 3).

Out of 110 measurable road kills, 97 (49 in 2003 and 48 in 2004) were below 30 cm,

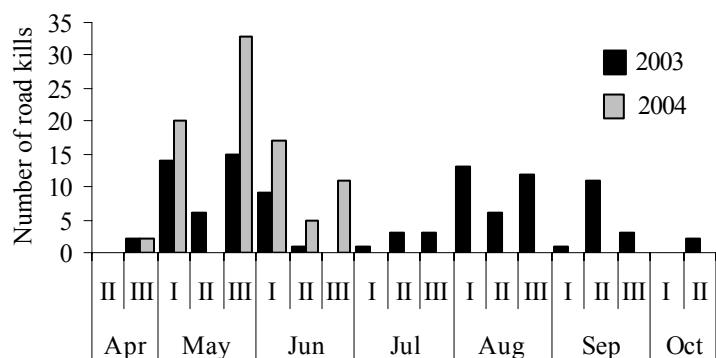


Fig. 2. Mortality dynamics of Grass snakes *Natrix natrix* ( $n = 190$ ) on the surveyed stretch of a suburban road (see Fig. 1). Roman numerals (I–III) mark consecutive 10-day periods in a month.

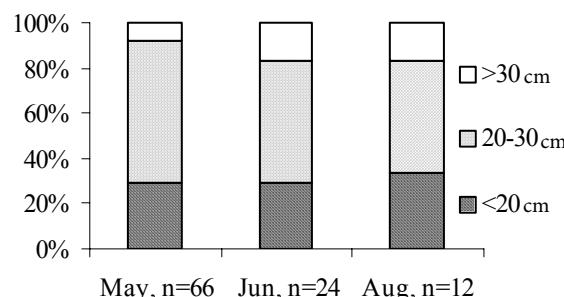


Fig. 3. Total body length (TL, in cm) distribution among Grass snakes *Natrix natrix* ( $n = 102$ ) found dead on the surveyed stretch of a suburban road (see Fig. 1) in the months with at least 10 road kills per month.

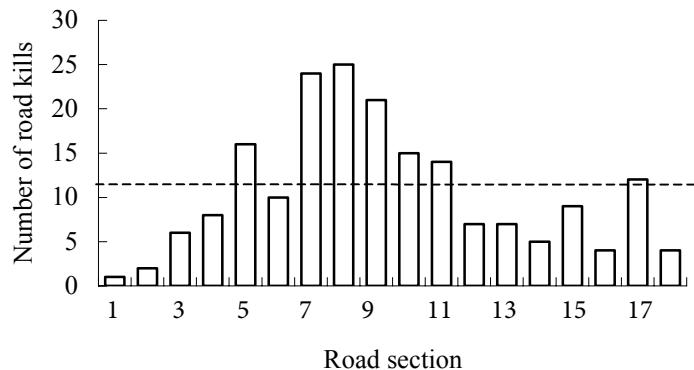


Fig. 4. Mortality distribution of Grass snakes *Natrix natrix* ( $n = 190$ ) among the 100-meter sections of the surveyed 1800-meter suburban road stretch (see Fig. 1). Broken line marks the mean of 10.55 road kills per section.

10 (5 in 2003 and 5 in 2004) ranged 30–50 cm, and 3 (2 in 2003 and 1 in 2004) were above 50 cm. Thus 89% of the measurable road kills were juveniles, with the subadults and adults contributing only 8% and 3%, respectively. The contribution of hatchlings remained approximately at the same level of ca. 30% throughout the season (Fig. 3), the slight increase in the August sample being insignificant (Fig. 3;  $\chi^2$  test,  $\chi^2 = 7.07$ ,  $df = 4$ ,  $P < 0.52$ ). However, the contribution of older juveniles decreased in June in favour of subadults (Fig. 3).

Dead grass snakes were found along all 18 road sections but their numbers varied between 1 and 25 (Fig. 4). The highest mortality was recorded within three road sections (7–9), where 37% of all road kills were found (Fig. 4). Real numbers of dead

snakes found along each road section clearly diverged from the mean of 10.55 snakes for a 100-meter section ( $\chi^2$  test,  $\chi^2 = 188.82$ ,  $df = 17$ ,  $P = 0.0001$ ; Fig. 4).

Regression analysis on habitat variables as determined for each road section confirms expectations based on the known habitat preferences of grass snakes: the road kill numbers are positively correlated with the length of woodland border, which accounts for 46% variability in road-kill numbers, and negatively correlated with the length of border to agricultural land (Table 1). The negative correlations to the nearest distances to the river and standing water bodies are nonsignificant but not far from the boundary value of  $P = 0.05$ , which makes them likely to be real.

The recorded road-kill numbers show a significant correlation with maximum tem-

Table 1. Regression analysis of the Grass snake *Natrix natrix* road mortality on habitat variables as determined for each of the eighteen 100-meter sections of the surveyed road (see Fig. 1).

Variable (range)	$\beta$	$R^2$	$B$	$F_{1,16}$	P-value
Forest edge (0–200 m)	0.68	46%	0.062	13.73	0.002
Field edge (0–200 m)	-0.68	46%	-0.062	13.73	0.002
Distance to standing water (40–400 m)	-0.37	14%	-0.023	2.62	0.125
Distance to river (200–760 m)	-0.41	16%	-0.019	3.18	0.093

Table 2. Spearman rank correlation coefficients ( $r_s$ ) between weather conditions and the number of Grass snake *Natrix natrix* road kills found on the surveyed stretch of road. The bottom figures refer to survey days, and the top figures to preceding days. Significance levels marked with asterisks: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Nonsignificant correlation marked as ns.

Year (number of surveys)	Mean daily temperature	Maximum daily temperature	Minimum daily temperature	Minimum daily near-ground temperature <sup>a</sup>	Daily rainfall
2003 ( $n = 49$ )	0.25 ns	0.33*	0.03 ns	-0.03 ns	-0.09 ns
	0.21 ns	0.29*	0.11 ns	0.12 ns	-0.13 ns
2004 ( $n = 28$ )	0.68***	0.70***	0.36*	0.29 ns	0.04 ns
	0.63***	0.57**	0.34 ns	0.26 ns	-0.16 ns
2003 and 2004 ( $n = 77$ )	0.31**	0.39***	0.12 ns	0.06 ns	-0.04 ns
	0.29*	0.34*	0.17 ns	0.16 ns	-0.13 ns

<sup>a</sup> Five cm above the ground.

peratures on both survey days and preceding days in the spring of 2004 (Table 2). This correlation is much weaker when calculated for the entire year 2003 and non-significant ( $r_s = 0.31$ ,  $n = 29$ ) when calculated for the same period (April 20–June 30) as in 2004.

The correlation with minimum daily temperatures (either the regular or the near-ground temperatures) proved to be non-significant except for a weak correlation with the preceding day minimum temperatures in 2004 (Table 2). This suggests that the majority of snakes perish during the warmest hours of the day. Also, there is no significant correlation with daily rainfall, suggesting little significance of humidity for the activity of grass snakes.

## 5. DISCUSSION

The obtained mortality estimates ( $1.16$  snakes  $\text{km}^{-1}$   $\text{day}^{-1}$ ,  $204$  snakes  $\text{km}^{-1}$   $\text{year}^{-1}$ ) are extremely high, many times higher than estimates obtained for longer road stretches. Our figure comes from a relatively short stretch of road that was selected because of the high road mortality of amphibians and thus cannot be representative of the grass snake road mortality over larger areas. On the other hand, our study area does not seem to be unique, at least for the region of Lower Silesia where many roads cross similar habitats, and only a part of the studied stretch crosses preferred grass snake habitats.

Several factors account for the high percentages of juveniles among the recorded road kills. First, the percentage of juveniles in the whole population is higher than, e.g., in the *Vipera* adders (Brito and Álvares 2004), because of oviparity and thus higher fecundity of the grass snakes. Second, the juveniles tend to disperse away from the hatchery, which contributes to their mortality (Bonnet *et al.* 1999) and the hatcheries were most probably present in the piles of decaying leaves in the wooded areas adjacent to the surveyed road. Consistently, the number of road kills correlates positively with the length of woodland borders (Table 1).

The majority of grass snakes are known to hatch toward the end of August (Juszczyk 1987, Günther and Völkl 1996, Luiselli *et al.* 1997) and thus the hatchling mortality should peak late in the summer (Bonnet *et al.* 1999). This makes the observed high (*ca.* 30%) hatchling mortality throughout the season of 2003 (Fig. 3) unexpected. In particular, the late spring mortality peak with the high contribution of hatchlings suggests a high frequency of spring hatching which is hitherto known to occur only sporadically in the grass snake (Juszczyk 1987). With the incubation period of about two months, the spring hatching necessitates either laying in the early spring or egg wintering. Since the pregnancy period is again about two months, the early spring laying would in turn necessitate the autumn mat-

ing, which was frequently observed (Günther and Völkl 1996, Kabisch 1999), and delayed fertilization (*amphigonia retardata*), which is known to occur in the grass snake (Kabisch 1999). Whatever the mechanism of extending the hatching period into the spring, it is likely to be facilitated by a combination of the global climate warming and the warm local climate of Lower Silesia. In Poland, the warming is marked by a significant increase (by 1.0 to 1.5°C) of the average winter and spring temperatures, which is accompanied by a slight cooling of the autumn (Kożuchowski 2003). The impact of the latter may be compensated by a high mean October temperature and the highest for Poland mean autumn temperature, both above 9°C for years 1971–2000 (Lorenc 2005).

Of all weather factors analysed, the maximum daily temperatures had the greatest impact on the road kill numbers (Table 2). Aside from the known positive correlation between the ambient temperature and activity (Mertens 1994), this result is consistent with the exclusively diurnal habits of grass snakes (at least on land), which are active mainly between 10:30 am and 5 pm (Kabisch 1999). In addition, the synchronised hatching on warm days (Luiselli *et al.* 1997) may have contributed to the observed correlation as the piles of decaying leaves, which were present alongside the studied road, may lead to communal laying to a single brood chamber (Eckstein 1993).

There are, however, marked differences between the two study seasons, the correlation with maximum daily temperatures being non-significant for the spring of 2003 but highly significant for the spring of 2004. The summer of 2003 was one of the three warmest over the last two decades, with record high temperatures of June (daily mean 20, maximum daily mean 26.4) and August (maximum daily mean 27.9). The summer of 2004 was much cooler, with temperatures in June (daily mean 17.1, maximum daily mean 22.9) close to the average and temperatures in May (daily mean 13.2, maximum daily mean 18.7) slightly below the average. This suggests that snakes' mobility is limited by maximum daily temperatures under the average and even more so under cool weather conditions.

Pavements are known to attract some reptiles because of being warmer than the surrounds (e.g., Ashley and Robinson 1996, Shine *et al.* 2004). However, we never observed any indication of thigmothermic behaviour on the studied road stretch and on several occasions we saw the snakes cross the road at right angles and a high speed. Shine *et al.* (2004) observed a similar road-avoiding behaviour in another naticine, *Thamnophis sirtalis parietalis*. In addition, the greatest temperature differences between the tarmac and the environment occur in the evening and at night (Shine *et al.* 2004), turning it to a potential hazard for nocturnal rather than diurnal reptiles.

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