

Repeated use of roadside tunnels of the European mole (*Talpa europea*) as a communal nesting area by grass snakes, *Natrix natrix*: are there thermal benefits?

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ABSTRACT - The presence of oviparous reptiles in relatively cool climates is mostly due to the ability of reproducing females to locate nest-sites with appropriate thermal regimes. In northern Europe the grass snake (*Natrix natrix*) is well known for exploiting anthropogenic derived heat sources to aid egg incubation. This paper documents, for the first time, *N. natrix* repeatedly exploiting roadside areas for egg-laying where females oviposited in tunnels of the mole *Talpa europea*. Temperatures in the tunnels were higher than dummy nest temperatures in shaded, partially shaded and sunlit areas around the nesting area but in good agreement with the temperatures of a dummy nest at a similar distance from the road edge. Hatching success in six clutches ranged from 87.5 – 100%. These results suggest a thermal benefit from heat from roadside tarmac for egg incubation.

INTRODUCTION

Unlike viviparous reptiles that have the capacity to track shifting thermal zones to facilitate the development of embryos, in oviparous species the eggs, once deposited, are immobile and subject to changes in local climatic, which could depart significantly from optimum. In addition, the influence of thermal regimes on offspring fitness is known to impact on nest site selection (e.g. Burger & Zappallorti, 1986; Rhen & Lang, 1995; Deeming 2004; Blouin-Demers et al., 2004; Brown & Shine, 2004; Brown & Shine, 2005; Löwenborg et al., 2010; Refsnider & Jansen, 2010; Löwenborg et al., 2011). This is particularly important in temperate regions, where weather changes are frequent and incubation may last from between a few weeks to months. When oviparous reptiles repeatedly use the same nesting sites in natural conditions, this suggests that appropriate conditions for incubation are present and hence information from such sites provides insight into the conditions that females actually select (Refsnider & Jansen 2010; Löwenborg et al., 2010).

The European grass snake, *Natrix natrix*, is an oviparous snake with a distribution extending further into northern Europe than any other oviparous snake (Street, 1979). It is known to select sites with rotting vegetation for oviposition (e.g Street, 1979; Gregory, 2009; Lowenborg et al., 2010; Baker, 2011). These generate warmth and often retain moisture, important factors in enabling successful incubation of eggs, especially in northerly latitudes. In a human landscape, compost heaps frequently serve this function, especially if they also have exposure to sunshine (Löwenborg et al., 2010). This paper describes thermal conditions inside roadside nest sites that were repeatedly used by *N. natrix* at a location in western France during 2014 and 2016.

METHODS

The nesting area was discovered during 2014 in sandy soil at a road-side grass verge 1.3km east of the village of Grues (46°39N;1°29W) in Department 85, Vendée, France. The verge was on a gradient of around 10 - 15° and faced south. The road was bordered by a hedgerow at a distance of around 1500mm from the road and small woodland surrounding a pond but most of the outer terrain was agricultural land (Fig. 1). The nests had apparently been raided by a species of small mammal (likely a mustelid based on scats found at the nesting area) with most egg remains scattered on the surface (Fig. 2). The eggs had been deposited in the tunnels of European mole (*Talpa europea*) that ran parallel to the road and hedgerow (Fig. 1). The absence of mole hills during summer suggests the tunnels were abandoned at this time. The nest location was marked (using paint at the road edge) and during 2016 a total of 63 visits made to the site from 17 May to 5 October, between 1130h and 1830h.

On each visit measurements of temperatures inside the tunnels were made using a *Koch Thermo-Hydrometer* (error $\pm 0.2^{\circ}\text{C}$). To give insight into nest site selection several dummy nests around the nesting area were excavated within 20m of the nesting area. This method has value in describing the distribution of possible nest temperatures available and can be compared with the temperatures in the true nesting area. This included 1) a sunny position at the roadside 32.8m east from the nesting area ($\text{Nest}_{\text{road}}$) 2) in full sun 19m from the roadside ($\text{Nest}_{\text{open}}$) 3) partially shaded area 21.9m from the roadside ($\text{Nest}_{\text{partial/shade}}$) and 4) at a shaded area 11.3m from the roadside ($\text{Nest}_{\text{shade}}$). The locations are shown in Fig. 1. The measurements in the dummy nests were made at a similar depth into the soil as the real nests. Road surface temperatures next to the nesting area were recorded using a Electronique Frontal infra red detector (model TS112). This provided a faster



Figure 1. Aerial view of the study area showing localities of the nesting location (dotted line at roadside), artificial nests (Sr = roadside in sun; S = sunny; Ps = partial sun; Sh = shade), pond (P) and location of a road-killed gravid female *N. natrix*. See text for further details. In this *Google Earth* view the pond next to the nesting area appears dried out, which is usually from June onwards.



Figure 2. Example of a roadside nest, one of the two nests located in 2014, which has been excavated by a predator

reading than the Koch instrument for use with passing traffic.

Statistical analysis. Two gravid females were found road-killed close to the site in late June and it is assumed that egg deposition occurred sometime in July and hatching during September. Subsequently 24 visits to the site were made from 3 July to 5 October and statistics were applied only to data from this period. On each visit measurements of temperatures at the nesting area and 4 artificial egg-laying sites were made. In addition, road surface temperatures adjacent to the nesting area were also taken. Therefore sample sizes in the analysis were $n = 24$

for each data set, i.e. egg laying site, artificial egg laying sites and road surface.

All \pm values represent standard deviations. Leven's test (L) was applied to determine homogeneity of variances with the null hypothesis $\sigma_1^2 / \sigma_2^2 = 1$ and $\alpha = 0.05$. Equal variances were found (L values 0.31 to 3.8, p from 0.06 to 0.58) in all data sets except between $Nest_1$ and $Nest_{shade}$, $L = 5.49$, $p = 0.02$. Temperatures were then compared using a non-parametric Friedmans repeated measures ANOVA, which employs a ranked procedure at $\alpha = 0.01$ (Gotelli & Ellison, 2004). The null hypothesis is sum of ranks equality for all sample groups. Post-hoc analysis was with a two-tailed non-parametric Wilcoxon signed-rank test giving a z -score with $\alpha = 0.01$.

RESULTS

A total of 38 empty egg shells were found in two clutches (N_1 and N_2) on 28 09 2014. The eggs had been deposited approximately 120mm ($nest_1$) and 90mm ($nest_2$) into the soil situated 170 ($nest_1$) and 140mm ($nest_2$) from the road edge at around 8 metres apart (Fig. 1).

Between 04 09 16 and 22 09 16, four clutches totalling 74 egg shells were found at the same location at distances between 360 and 410mm from the road edge. They were at similar depths to the 2014 sites at around 120mm into the base of the tunnels. This gave a total, for both years (2014 and 2016), of 112 eggs. During 2016 numerous shed skins were found inside the tunnels alongside 2 emerged but dead fully formed hatchlings at the opening of N_3 , and 2 full term embryos still within the eggs in N_4 . Egg remains at two

additional nests were observed on 18 09 16 and 22 09 16 (N_5 and N_6) and indicated all hatchlings emerged successfully from these. The data are summarised in Table 1.

Table 1. Egg clutch information from 2014 and 2016; * indicates 2 dead fully formed hatchlings outside shell and ** 2 full term hatchlings dead in the shell. Measurements are in mm.

Nest	Date	Clutch Size	Egg Length	Egg Width	Distance from road edge (mm)	Hatching success rate (%)
N_1	28/09/14	28	28	18	170	100
N_2	28/09/14	10	25	15	140	100
N_3	04/09/16	26	29	15	360	92.3*
N_4	04/09/16	16	31	20	410	87.5**
N_5	18/09/16	17	29	19	370	100
N_6	22/09/16	15	26	19	365	100

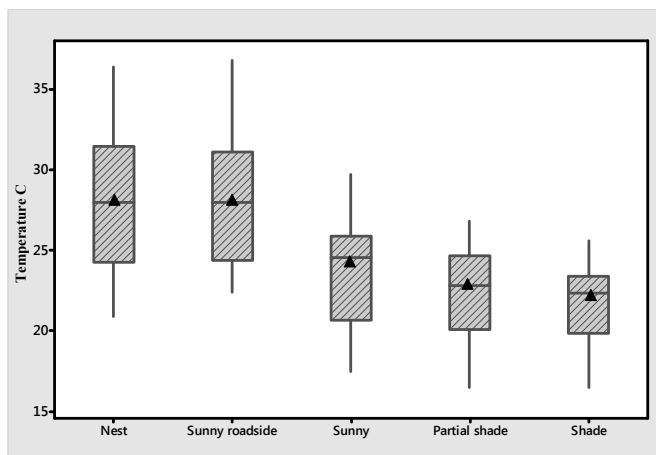


Figure 3. Boxplots of temperatures (°C) inside the nesting area and those of artificial nests. Triangles represent means, boxes the interquartile ranges, horizontal lines within the boxes medians and vertical lines the ranges.

During sunny weather road surface temperatures next to the nesting area ($mean = 43.5 \pm 9.4^\circ C$) were higher than when overcast ($mean = 37.5 \pm 8.5^\circ C$). The Friedman’s test revealed significant differences between the data sets, $\chi^2 = 28.45$, $p < 0.001$, $d.f. = 4$. Post-hoc analysis indicated no significant difference between the nest area ($mean = 28.2 \pm 4.3^\circ C$) and Nest_{road} ($mean = 27.9 \pm 3.9^\circ C$; $z = 0.36$, $p = 0.72$) but nest area was significantly higher than Nest_{open} ($mean = 23.7 \pm 3.2^\circ C$; $z = 3.8$, $p < 0.01$), Nest_{partial/shade} ($mean = 22.5 \pm 2.7^\circ C$; $z = 4.29$, $p < 0.01$) and Nest_{shade} ($mean = 21.8 \pm 2.5^\circ C$; $z = 4.28$, $p < 0.01$). The data are shown in Fig. 3.

DISCUSSION

Several oviparous snake species have been reported nesting adjacent to roads (e.g. *Pituophis melanoleucus*; Burger & Zappalorti, 1986; *Zamenis longissimus*; Kovar et al., 2016) but this is the first report of the behaviour in *N. natrix*. In *N. natrix* a likely explanation was the consistently higher temperatures present at the nesting area due to heat from roadside tarmac that produced

mean temperatures in the nesting area within the optimum of $27 - 29^\circ C$ for development found for *N. natrix* in laboratory studies. These temperatures have produced early hatching and large offspring capable of enhanced locomotor performance (Löwenborg et al., 2010; Löwenborg et al., 2011; Hagman et al., 2012). However, the observed temperature range at the site was $20.9 - 36.4^\circ C$, and would have been much wider had there been nocturnal measurements. In laboratory studies fluctuating temperatures produced higher incidences of scale abnormalities in *N. natrix* compared to embryos incubated at constant temperatures and have been associated with low survivorship in adults (Löwenborg et al., 2012). Fluctuating temperatures are likely normal in natural nest sites but Löwenborg et al., (2012) suggested that the amount of time temperatures remain at either end of the thermal spectrum may be a key factor that impacts on embryonic development. Egg slit data (an indicator of hatching success; e.g. Doody et al., 2015), suggested high rates of hatching success under fluctuating temperatures (Table 1), but no data are available on scale abnormalities. It is worth noting that female viviparous snakes thermoregulate precisely when gravid, which produces larger fitter offspring and fewer stillborn. (e.g. Arnold & Peterson, 2002; Lourdaïs et al., 2004) but at a cost of increasing risk to predation as a consequence (Lourdaïs et al., 2004).

Communal nesting and repeated use of nest sites has been frequently reported in snakes (e.g. Foley, 1971; Covacevich & Limpus, 1972; Brown & Shine, 2005; Braz et al., 2008) with the presence of egg shells from previous nestings cited as the main cue for nesting area location (e.g. Plummer, 1981; Brown & Shine, 2005). Temperatures in communal nests have found to be higher than in solitary nests, which enhances hatchling fitness (e.g. Blouin-Demers et al., 2004) but other benefits such as predator satiation may reduce egg mortality. However, many species of snake may be unable to construct a nest and rely on pre-existing sites for oviposition (Packard & Packard, 1988). In this respect the tunnels of *T. europaea* in sandy soils present several potential advantages as nesting environments as *T. europaea* normally avoid tunneling in soils that flood or are excessively dry (Funmilayo, 1977). Sandy soils heat up more rapidly than clay based soils (which are common in the area) and drain rapidly whilst also retaining moisture (Townsend, 1977). These are soil characteristics that facilitate reptile egg incubation. Soils with good moisture retention could be especially important where summers are hot and dry since humidity has been identified as an additional key factor influencing both hatching success and phenotypic traits in reptiles (e.g. Packard & Packard, 1988; Löwenborg et al., 2012) although may not be as important for reptiles as temperature (Warner & Andrews, 2002). Humidity in the nesting area between 3 July and 2 October ranged from $27 - 85\%$ ($mean = 61.7 \pm 17.5\%$) and gradually declined during the incubation period.

No nests were found in 2015 (this does not of course indicate there were none) but the two gravid females (TL 680mm and 810mm) found road killed close to the site (170 and 480m) during June 2015 could have been migrating towards the nesting area. If the snakes originated

from wetlands due east and north of the nesting area the fragmented nature of the surrounding landscape, which consisted mostly of agricultural land, this would necessitate movement through hedgerow corridors (Fig. 1). This would also involve road crossings and mortality risk from road traffic and predators (Meek, 2015) but presumably the benefit of reaching the nesting area outweigh the risks.

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