Interpreting dispersal patterns of reproductive female *Hierophis viridiflavus* (Lacépède, 1789), around a communal nesting site (Squamata: Serpentes: Colubridae)

Zur Verteilung weiblicher *Hierophis viridiflavus* (Lacépède, 1789) um einen gemeinschaftlichen Eiablageplatz in Abhängigkeit von ihrem Trächtigkeitsstatus (Squamata: Serpentes: Colubridae)

Lorenzo Rugiero & Massimo Capula & Leonardo Vignoli & Luca Luiselli

**ABSTRACT**

Using data acquired over 17 years, dispersal patterns around a communal nesting site (CNS) of adult females of the oviparous colubrid snake *Hierophis viridiflavus* (Lacépède, 1789), were investigated. The authors analyzed capture-mark-recapture data in terms of the distances at which adult females were found from their CNS i.e., oviposition place. The data revealed the following:

1. Some reproductive females seem to be able to switch between the options of ovipositing at the CNS or elsewhere.
2. Females who oviposited at the CNS in a given year were by trend observed at short distances from this oviposition site in the year before the effective nesting.
3. Females of poor body condition after oviposition engaged in longer displacements from the communal nesting site, possibly to increase foraging efficiency and rebuild lost body reserves prior to entering brumation.

**KEY WORDS**

Reptilia: Squamata: Serpentes: Colubridae, *Hierophis viridiflavus*; communal nesting, movement behavior, Central Italy

**INTRODUCTION**

Recent studies of spatial ecology in free-ranging snakes revealed intriguing biological phenomena (e.g., see Shine et al. 2001; Carfagno & Weatherhead 2006) including the experimental demonstration of patterns previously supposed e.g., that snake dispersal is male-biased (Keogh et al. 2007).

Adult female snakes may exhibit unusual movement patterns because of their high ‘costs’ of reproduction (e.g., Shine 1980; Madsen 1987; Bonnet et al. 2001). For example, gravid females may engage in long distance displacements to find suitable oviposition sites, which are scarce in cold climate regions (e.g., Madsen 1983, 1987).

Females of several species may oviposit in communal nesting sites (e.g., Gordon & Cook 1980; Gomille 2002) where they may return to lay eggs several times during their life (Filippi et al. 2007; Luiselli et al. 2011).
Study species.- The Western Whip Snake, *Hierophis viridiflavus* (LACÉPÈDE, 1789) is a medium-sized (up to 150 cm long), oviparous, colubrid snake that feeds mainly upon lizards and small rodents (RUGIERO & LUISELLI 1995; ELIEVRE et al. 2012). At the study area, this snake is above-ground active from March to November, and most of the females breed every year (FILIPPI et al. 2007; LUISELLI et al. 2011). The authors present a study on the movement patterns of female Western Whip Snakes for years in which they were not present at the communal nesting site. In the study, about half of the females inhabiting the study area came back year by year, or at least in alternate years, to lay eggs at the communal oviposition site (CAPULA & LUISELLI 1995; FILIPPI et al. 2007), whereas the other half was observed only once ovipositing at the communal nesting site (see Appendix 1 in LUISELLI et al. 2011). A reasonable assumption explaining this latter pattern implies that some females are unable to breed annually due to high energy costs of reproduction, (e.g., BONNET et al. 2001) and hence would not return to the communal oviposition site in such years. An alternative hypothesis is that the 'missing' females laid their eggs in places different from the communal nesting site.

In the present paper the authors examined which of the two above-mentioned hypotheses applies, and in addition, tested whether a female snake remains close to, or at some distance from, the communal nesting site in the years in which she does not oviposit there.

MATERIALS AND METHODS

Study species.- The Western Whip Snake, *Hierophis viridiflavus* (LACÉPÈDE, 1789) is a medium-sized (up to 150 cm long), oviparous, colubrid snake that feeds mainly upon lizards and small rodents (RUGIERO & LUISELLI 1995; ELIEVRE et al. 2012). At the study area, this snake is above-ground active from March to November, and most of the females breed every year (FILIPPI et al. 2007; LUISELLI et al. 2011). The authors present a study on the movement patterns of female Western Whip Snakes for years in which they were not present at the communal nesting site. In the study, about half of the females inhabiting the study area came back year by year, or at least in alternate years, to lay eggs at the communal oviposition site (CAPULA & LUISELLI 1995; FILIPPI et al. 2007), whereas the other half was observed only once ovipositing at the communal nesting site (see Appendix 1 in LUISELLI et al. 2011). A reasonable assumption explaining this latter pattern implies that some females are unable to breed annually due to high energy costs of reproduction, (e.g., BONNET et al. 2001) and hence would not return to the communal oviposition site in such years. An alternative hypothesis is that the 'missing' females laid their eggs in places different from the communal nesting site.

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radius), the CNS being situated in the center (Luiselli et al. 2011). In the core area, field effort per year was at least 12 days (hrs 0800-1800) in 1990-1997 and in 2001, and at least 24-28 days (hrs 0800-1800) in 2002-2009, with three people independently searching for snakes each day. Thus, the overall field effort was approximately 360 person hours per year in 1990-1997 and in 2001, and 720-840 person hours per year in 2002-2009. Additional searches, more irregularly distributed across the year, were also performed in the peripheral area. The whole area surveyed, including both the core and the peripheral, was 9.3 ha (that roughly can be visualized by a circular area of about 172 m radius), with the CNS being situated approximately in the center.

Several observations of marked snakes included in this study refer to individuals spotted considerably outside the core and peripheral areas, which are part of a region employed by the authors for more than 20 years of studying the ecology and demography of *Vipera aspis* (Linnaeus, 1758) (e.g., see Rugiero et al. 2012).

Snakes were captured by hand and individually marked by ventral scale clipping (Brown & Parker 1976) for future identification. Females were palpated in the abdomen to verify their pregnancy status and to count the number of eggs according to the method outlined in Filippi et al. (2007). Date of capture and both weight and length (SVL, precision ± 0.5 cm) were recorded.

The precise site of capture of each female was entered on a digital map using GPX, and its linear distance from the CNS was calculated by DIVA-GIS free software available at http://www.diva-gis.org/ (precision ± 1 m). For homogeneity across individuals, only one (the first) capture per individual and year was considered.

Due to the small size of the stone building used as CNS by snakes and the high field effort concentrated at the oviposition period (mostly end of June to mid-July; in later years chiefly in late June), it is likely that in all study years we captured the great majority (if not all) of the females reproducing at the CNS (Filippi et al. 2007; Rugiero, Capula, Vignoli & Luiselli, unpublished).

**Statistical analyses.** Only those females (*n* = 57) entered the analyses which (i) had oviposited at the CNS at least once within the study period (see Luiselli et al. 2011) but (ii) were not observed to arrive at the CNS for oviposition in the year when they were captured (*n* = 28; this study). The records were divided in three categories:

1. **marked females captured outside CNS while gravid in a given year and did not oviposit (i.e., were not observed) at CNS in that same year, and thus, were likely to have laid eggs at unknown sites different from CNS (type 1 females, *n* = 8). Occurrence of type 1 females documents that these individuals did not oviposit obligatorily at the CNS, although many of these snakes regularly returned to the CNS for oviposition in the study years (Filippi et al. 2007; Luiselli et al. 2011);**

2. **marked females captured outside CNS while non-gravid in a given year, but laid eggs (i.e., were observed) at the CNS in the following year (type 2 females, *n* = 12)**

3. **marked females recaptured outside CNS in non-gravid status in the year following the year of oviposition at CNS (type 3 females, *n* = 8). Females of the latter categories could indicate migratory tendencies of snakes that have just laid eggs at the CNS (type 3 females) or that are going to lay eggs at the CNS (type 2 females) in the year following/preceding egg-laying at the CNS.**

The authors compared the linear distances from the CNS of the sites at which type 2 and 3 females were found, to test whether the movement patterns adopted by a female in a given year (i.e., staying closer to, or farther from, the CNS) could predict her nesting activity in the following year.

Nonparametric statistics were used when normality (tested by Kolmogorov-Smirnov test) was not achieved in the examined variables. The differences in the mean distances from the CNS between type 2 and type 3 individuals were tested by Mann-Whitney U-test, and those between the three categories of individuals by Kruskal-Wallis ANOVA.

Logistic regression analysis was applied to discern whether or not the linear distance of capture from the CNS, recorded in a given year, would predict the occurrence of oviposition at the CNS in the fol-
Results

Overall, eight type 1 females were captured, sighted at 319.1 ± 146.8 m from the CNS (median = 391; range: 73-445). The sample size is too small to reveal whether there was an effect of the year on the probability of recording individuals of this category at the study area. The authors collected distance data for 12 type 2 and eight type 3 females. Distance ranges were 15-211 m in type 2 (median = 72.5), and 71-445 m in type 3 (median = 209.5) (Table 1). The ranges of type 1 and 3 females were almost identical, whereas their medians differed clearly. There was a clear trend for type 2 individuals to be captured closer to the CNS than individuals of types 3 and 1 (Fig. 1). Indeed, the average distances from CNS at capture of type 2 females (89.2 ± 65.7 m) were significantly lower than those of type 3 females (220.6 ± 123.5 m) (Mann-Whitney U-test: $Z = -2.733$, $U = 13$, $P < 0.005$). There were significant differences between females of types 1, 2 and 3 as for their distances of capture from the CNS (Kruskal-Wallis ANOVA: $H = 11.45$, $P = 0.00327$), with type 2 females being found at significantly lesser distances than both type 3 and 1 females, the latter being captured at similar distances from the CNS (Kruskal-Wallis ANOVA: $H = 2.01$, $P = 0.1559$).
Table 1: Information on 28 female *Hierophis viridiflavus* (Lacépède, 1789) captured and recaptured in the study area (Oriolo Romano, Viterbo, Italy). The snakes satisfy the selection criteria of (i) having oviposited in the communal nesting site (CNS) at least once within the study period and (ii) were not observed to arrive at the CNS for oviposition in the year when they were captured.

Tab. 1: Daten zu 28 im Untersuchungsgebiet (Oriolo Romano, Viterbo, Italien) gefangenen und wiedergefangenen Weibchen von *Hierophis viridiflavus* (Lacépède, 1789), die folgenden Auswahlkriterien genügten: (i) ihre Eiablage mußte innerhalb des Untersuchungszeitraumes zumindest einmal am gemeinschaftlichen Eiablageplatz (CNS) erfolgt sein und (ii) die Tiere durften im Jahr ihres Fanges nicht am gemeinschaftlichen Eiablageplatz angetroffen worden sein.

<table>
<thead>
<tr>
<th>Snake ID</th>
<th>Category</th>
<th>Capture date</th>
<th>Gravidity status</th>
<th>Recapture date</th>
<th>Gravidity status</th>
<th>Body length (cm)</th>
<th>Linear distance from CNS (m)</th>
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<td>June 1995</td>
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<td>+</td>
<td>June 2009</td>
<td>-</td>
<td>97.5</td>
<td>71</td>
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A logistic regression model (\(-2 \log \text{Likelihood} = 17.992\); Goodness-of-fit = 16.537; \(\chi^2 = 9.533, df = 1, P < 0.002\)) revealed that the distance from CNS at a given year was a good predictor of the occurrence of oviposition at the CNS in the following year (80% of cases correctly classified; \(\beta = -0.194, r = -0.328, P = 0.026\)).

**DISCUSSION**

The capture-mark-recapture data showed that:

(i) some gravid female *Hierophis viridiflavus* that oviposited at least once at the CNS did not obligatorily do so over the years and must have oviposited also in places different from the CNS (type 1 females);

(ii) these type 1 females were usually found at considerable distances from the CNS;

(iii) females which oviposited at the CNS in a given year tended to be found at short distances from the CNS in the year before (type 2 females); and

(iv) females captured in non-reproductive state in the year after oviposition at the CNS (type 3 females) were more frequently observed comparatively far from the CNS.

Concerning item (i), it is unknown what makes a female snake select on whether to oviposit at the CNS or elsewhere. Results from items (iii) and (iv) indirectly suggest that many females ovipositing at the CNS do not engage in long displacements finding their nesting site, as observed in other oviparous snakes, including e.g., Grass Snakes *Natrix natrix* (Linnaeus, 1758), from Sweden (Madsen 1984, 1987). These patterns are also confirmed by the logistic regression analysis clearly showing that distance of capture from CNS of a given female in a given year predicts her oviposition at the CNS in the following year.

We have no direct evidence of the reasons behind the fact that type 3 females were found at distances from the CNS significantly greater than observed in type 2 females. We hypothesize that those females are in poor body condition after oviposition (type 3) because of the high costs of reproduction (Shine 1980; Madsen 1987; Bonnet et al. 1999, 2002) and thus engage in longer displacements from the CNS in order to increase foraging efficiency, thereby rebuilding lost body reserves prior to brumation. The above-mentioned hypothesis is based on the finding, at the same *H. viridiflavus* population, that hatchlings in poor body condition were found at a longer distance from their birth place than heavier ones (Rugiero et al. 2012). They probably moved away to increase their foraging efficiency, a strategy that paid off well for the one-year old hatchlings that were pretty light at birth (Rugiero et al. 2012).

The hypothesis that the more emaciated post-oviposition females (i.e., those which were unlikely to reproduce again in the year following oviposition) moved farther away from the CNS than the less emaciated ones, is in agreement with the results of this study (type 3 females). Accordingly, these females would not reproduce every year, but only every second or third year, and, during the non-gravid period, accumulate energy to be invested in reproduction in the following years. This pattern is classic in temperate zone snakes, especially when trophic resources are not abundant (e.g., Shine 1980; Capula & Luiselli 1994; Gregory et al. 1999; Bonnet et al. 2002). Moreover, the Western Whip Snake has very high energy requirements (see Lelièvre et al. 2010b).

In the study area, 66.7% of the female individuals were recaptured while laying at the CNS in consecutive years (Filippi et al. 2007) confirming an annual reproduction frequency, and only 33.3% of the females visiting the CNS did so in alternate years (Filippi et al. 2007), apparently being compatible with the hypothesis of a biannual reproductive frequency (note that however these estimates are partially biased by the type 1 females, which were gravid but did not reproduce at the CNS in all years).

Other studies conducted in the same region confirmed that most females of the
Western Whip Snake lay eggs annually (frequency of annual breeders estimated to be at about 75 % of the total number of adult females; Capula et al. 1995), with considerable within-population individual variation due to the different body condition of post-reproductive females (Capula & LuiSelli 1994). Concerning the dietary spectrum of H. viridiflavus in central Italy (see Capizzo & LuiSelli 1996), there are only two potential prey species at the CNS, wall lizards Podarcis muralis (Laurenti, 1768), and rats (Rattus rattus) (see Filippi et al. 2007) and it is very likely that snakes remaining at the CNS after oviposition may have little opportunity to rebuild their depleted body fat reserves. Hierophis viridiflavus is an active hunter who can make important movements to feed (LeLievre et al. 2010a), so it is very likely that distance traveled may be correlated to hunting success.

In conclusion, the analysis of long-term data revealed a scenario where the advantages provided by the stone building for communal reproduction of snakes (i.e., good incubation conditions and low predation risks; see Filippi et al. 2007) are partially counterbalanced by the costs of the relatively low prey availability. Depending on their own body condition, the female strategy shifts between repeated oviposition at the CNS (strategy used by females in good body condition) to occasional use of the CNS and reproduction in alternate years (used by females in suboptimal body condition). These latter females would be forced to search for food in sites where trophic resource availability is higher than at the CNS.

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