Are communal nesting counts as useful as mark–recapture data for estimating population size in snakes?

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Snakes are rather difficult subjects for demographic studies. When snakes are not abundant in the field, herpetologists have learnt that a good method for population studies is to rely on mass captures at den sites. In several snake species females also exhibit oviposition at communal nest sites, which are utilized year after year. These oviposition sites may then serve to record individuals for snake population studies. Here, we compared population size estimates generated from a 17-year study of gravid females at a communal nesting site (CNF) with population size estimates from the same snake population across an 8-year traditional capture–mark–recapture (CMR) study. Although in our case only open population methods are appropriate for calculating yearly population sizes, we also used closed population methods in order to highlight an eventual effect of the models used. As a study species, we used the European whip snake (*Hierophis viridiflavus*) at a site in Mediterranean central Italy. Overall, population size estimates were significantly different between the two methods, with estimates from the CNF samples always higher than those obtained with traditional CMR. This difference was particularly strong with closed population methods, but still evident with open population models when the whole study period was considered. However, there were no statistical differences between population sizes estimated with CNF and CMR when only a subset of years (2002–2009) was used. No statistical relationship between population size estimates with CMR against CNF by year was uncovered, showing that CNF samples did not capture inter-annual variations in population sizes. We conclude that it might not be sound to use population size estimates from CNF samples instead of more traditional CMR studies, although yearly population size variations may at least in part be responsible for the differences between CNF and CMR estimates.

Key words: comparative analyses, demography, field techniques, Italy, Serpentes, statistics

INTRODUCTION

As snakes are elusive animals, often occurring at low densities, they are relatively intractable subjects for demographic studies. When snakes are not abundant in the field, herpetologists have learnt that a good method for population studies is to rely on mass captures at denning areas (e.g. Parker & Brown, 1972; Fitch, 1989). Indeed, several long-term studies on snake population ecology have been based on mass captures of individuals when snakes aggregate near their dens (e.g. Luiselli, 1995; Shine & Mason, 2001, 2004), which may serve for over-wintering and as location for mating (e.g. Madsen & Shine, 1992, 1993, 1994). However, hibernation and mating are not the only aggregation phases of the annual activity cycle for many snake species. For instance, females may oviposit at a communal nesting site, often located in anthropogenic structures (e.g. old buildings or manure heaps; see Madsen, 1984, 1987; Filippi et al., 2007). These human-made structures may offer optimal nesting sites because they are on average warmer than the surrounding landscape (Löwenborg et al., 2010). So far, communal nesting sites have been observed in several snake species from North America (Fitch, 1958; Gordon & Cook, 1980; Plummer, 1981), South America (Albuquerque & Ferrarezzi, 2004; James & Henderson, 2004), Africa (Rasmussen, 1993), and in the European species *Natrix natrix* (Matheson, 1962; Kabisch, 1974; Lapini,

1983; Madsen, 1984), *Zamenis longissimus (= Elaphe longissima)* (Lapini, 1983; Gomille, 2002), and *Hierophis (= Coluber) viridiflavus* (Filippi et al., 2007).

Although the ecology of communal nesting has not been very well explored, there is clear evidence that the same communal nesting sites may be used for many years (Capula & Luiselli, 1995; Filippi et al., 2007), and may thus serve as good sources for the mass captures of individuals for studies of population ecology. In addition, gravid females are slower escapers than non-gravid individuals, bask more often, and hence are more easily encountered and caught (this is especially true for fast species such as whip snakes). Even more importantly, female communal nesting aggregations have been observed in both temperate (e.g. Fitch, 1958; Gordon & Cook, 1980) and tropical snakes (e.g. Albuquerque & Ferrarezzi, 2004; James & Henderson, 2004), whereas aggregations at hibernacula or for the early spring mating season only occur in hibernating temperate zone snakes. However, despite these advantages, communal nesting sites have so far never been used for studies of population ecology.

For this paper we studied a population of western whip snakes, *Hierophis viridiflavus*, at a communal oviposition site in central Italy for 17 years (see Capula & Luiselli, 1995, and Filippi et al., 2007, for data on the early years of monitoring). *Hierophis viridiflavus* is a medium-sized (up to 150 cm long), oviparous, mainly lacertophagous colubrid, which is active from March to November in the

study area (Capizzi & Luiselli, 1996). We calculated population size and compared the population size estimates generated from the gravid female sample at the communal nesting site (CNF sample) with population size estimates based on randomly captured, marked and recaptured snakes (CMR sample) at the same study area over a multiyear period. As a test of the potential for using communal nesting females as a source of robust data for estimating population size in snakes, we assessed whether or not the estimates generated by counts of gravid females and by traditional CMR population surveys were consistent.

MATERIALS AND METHODS

Study area
The field study was conducted at Oriolo Romano (Province of Viterbo, about 400 m above sea level), 60 km north of Rome. The site was characterized by a partially dilapidated building (a stony box measuring 5.0 x 3.5 m, height 5 m) bordered by rich thorny vegetation (mainly *Rubus* sp.) and surrounded by cultivated fields. The climate of the study area is Mediterranean, with hot, dry summers, cool, wet winters and mild, wet springs and autumns (Tomaselli et al., 1973).

Field protocols
To capture gravid females at their communal nesting site, the surroundings of the dilapidated building were surveyed annually between 10 and 30 June in 1990–1997 and 2001–2009. Each year, at least 12 days were spent in the field (each field-day lasting from 0800 to 1800). Each field survey was done by two or three people who searched intensively for snakes throughout the study area. Since the study area was small (i.e. the dilapidated building plus a boundary area of about 100×80 m) and gravid females were easily located, we were able to achieve an adequate sample of individuals. Snakes were captured by hand, and individually marked by ventral scale clipping for future identification. Females were palpated in the abdomen to verify their pregnancy status and to count the number of eggs (see Filippi et al., 2007, for the data on the reproductive biology of these snakes).

In order to calibrate the population size estimates based on captures of communal nesting females, we performed a capture–mark–recapture (CMR) study of *H. viridiflavus* in March–April 2002–2009. Field effort per year was practically identical to that devoted to catching gravid females at the nesting site: each year we spent 12–16 days in the field (0800–1800), and three people searched for snakes each day. The size of the area used for the CMR study was 3 ha, the communal nesting site being situated in the centre. This size was selected on the assumption that the communal nesting site should be within the average home range of female whip snakes, which at the study area was 2.94±1.41 ha, with a range of 0.75–5.1 (*n*=16 radiotracked individuals, each one monitored for a period of 122–311 days, in the years 1993–2007). An estimate of approximately 3 ha as the average home range of a whip snake was also consistent with home ranges recorded by Ciofi & Chelazzi (1994) in another area of central Italy (home ranges of 1.2 to 5.1 ha, with females

having smaller home ranges than males). However, in central France whip snakes had larger home ranges than in our study area (7.74±2.1 ha, with female home ranges being 5.23±4.9 ha; Lelièvre, 2010). For this study, only adult snakes were considered, determined by the dorsal coloration livery of *H. viridiflavus*, which is considerably different between immature and mature individuals (Bruno & Maugeri, 1990).

Statistical analyses
Capture–mark–recapture analyses for estimating the abundance of our snake population were performed using methods for both closed and open populations. A population is defined as "closed" when it has a fixed size over the period of study, with no death, birth, immigration or emigration within the study area (Jolly, 1965, 1982). This assumption offers advantages, but can only be justified if the study is undertaken over a brief period of time. Therefore, open population methods are clearly more reliable in our case. However, we also used closed population methods to enable comparisons between population size estimates generated with the two methods. Although we consider our closed population estimates unreliable, they may still be useful in comparing the results obtained using CNF and CMR samples.

Assuming closed populations, we used the Petersen–Schnabel method. This method is based on the Petersen method (or Lincoln index), a simple mark-and-recapture method based on a single episode of marking animals and a second single episode of recapturing individuals. Schnabel (1938) extended the Petersen method to a series of samples. We estimated population size using 1) the Schnabel estimator, 2) the Chao estimator taking temporal change in capture probabilities into account, and 3) incorporating both individual and temporal differences in capture probabilities. Differences in catchability among individuals were assessed by the zero-truncated Poisson test.

As the open population method, we used a full Jolly–Seber model (Jolly, 1965, 1982; Seber, 1982). The basic equation in Jolly's method is:

$$N_i = (M_i \times n_i)/r_i$$

where N_i = the estimate of population in year i, M_i = the estimate of the total number of marked animals in the population in year i, r_i = the total number of marked animals recaptured in year i and n_i = the total number captured in year i. The proportion of marked snakes in the population at the moment of capture in year i is:

$$\alpha_i = r_i/n_i;$$

and the total population estimated for each year is:

$$\hat{N} = \hat{M}_i/\alpha_i.$$

The probability that a snake alive at the moment of release in the ith year will survive until the time of capture of the $i + 1$th year is:

$$\Phi_i = M_{i+1}/\hat{M}_i - r_i - n_i$$

Survival estimates slightly over one (e.g. in 1992, 1995 and 2005 in the CNF study, and in 2003 in the CMR study) may arise from sampling effects. When marks have been lost or were not recognized, a loss rate (the effect of death and emigration) can be estimated as:

$$\hat{\gamma}_i = 1 - \hat{\Phi}_i.$$

The number of new snakes joining the population in the interval between the ith and $i+1^{th}$ years and alive in year $i+1$ is given by:

$$^\wedge B_i = {}^\wedge N_{i+1} - {}^\wedge \Phi_i ({}^\wedge N_i - n_i + \alpha_i).$$

This may be converted to the dilution rate β:

$$(1/\beta) = 1 - ({}^\wedge B_i / = {}^\wedge N_{i+1}).$$

More details of these algorithms are given in Simply Tagging (2007).

In order to calculate population sizes for whip snakes based on CNF counts, we multiplied the estimates obtained by two, on the assumptions that all females in the population were gravid each year (i.e. reproduction was annual) and that the adult sex ratio was 1:1. Both these points were demonstrated by field data in early studies (Capula et al., 1995, 1997).

All demographic analyses were performed using Simply Tagging (version 1.31) software (Henderson & Seaby, 2002). The demographic analyses performed assumed constant natality, and conflation of survival and emigration. For density estimation, the area utilized by the population sampled was assumed to be 3 ha.

Other analyses were performed with Statistica (version 6.0) software. Parametric tests were used only after having verified data normality and homoscedasticity (Zar, 1984). Among-year differences in probability of capture of the individuals were calculated by one-way ANOVA followed by the Tukey HSD post-hoc test for paired comparisons. Sex-ratio departure from 1:1 was tested using χ^2 tests. A Mann–Whitney U-test was used to compare median numbers of years in which individuals were recaptured between CNF and CMR studies. A Wilcoxon matched pairs test was used to compare population estimates calculated with different methods for both CNF and CMR studies. Alpha was set at 5% (Zar, 1984). Means are followed by standard errors.

RESULTS

CNF study

Overall, 57 gravid females were captured during 17 years of study (Appendix 1). Their distribution of capture histories shows that the majority of individuals were recaptured 1–3 times, in up to three different years (Fig. 1A). Female population size, calculated on the assumption of a closed population model and a constant probability of capture among years, was 80±11.5 individuals (95% confidence intervals: 67–115).

The population size was 78±8.5 (68–102) using the Chao estimator. The yearly probability of capture ranged from 0.051 (in 2001 and 2004) to 0.128 (in 2008). According to the maximum likelihood Petersen–Schnabel census for temporal differences in capture probabilities, population size was 71±5.4 (95% confidence intervals: 65–86). There were substantial differences in the among-year probability of capture, with the years 2006–2009 showing a higher probability of capture than earlier years. Dividing the years into groups (group 1: 1990–1993, group 2: 1994–1997, group 3: 2001–2004, group 4: 2005–2009), there were significant differences among groups (one-way ANOVA, $F_{3,13}=3.955$, $P=0.033$), and a Tukey HSD post-hoc test revealed that capture probabili-
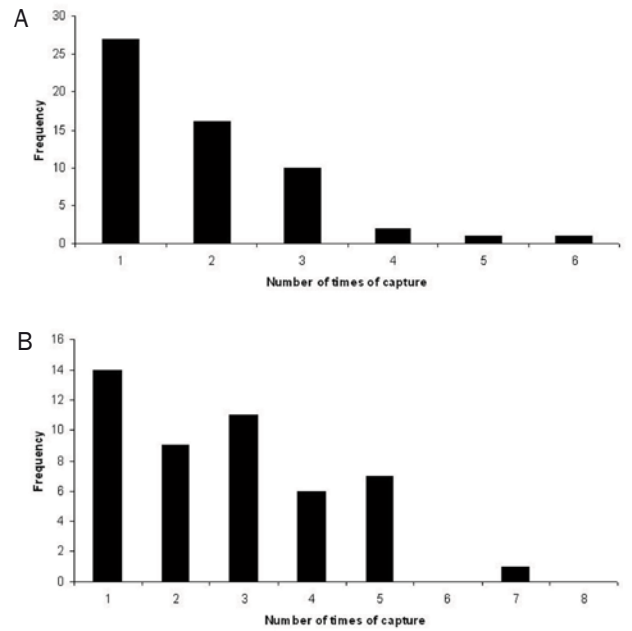

Fig. 1. Distribution of capture histories in gravid *H. viridiflavus* at the communal nesting site in 1990–1997, 2001–2009 (A) and in adult *H. viridiflavus*, during the CMR study in the years 2002–2009 (B).

ties in group 4 were significantly higher than in all other groups ($P<0.026$). The same trends were also seen using the maximum likelihood Petersen–Schnabel census (statistics not shown for brevity). The zero-truncated Poisson test for equal catchability among individuals showed that there were no inter-individual differences in catchability ($\chi^2=1.235$, df=2, $P=0.539$). A model incorporating both temporal and individual variation in capture probability gave a population estimate of 57±2.21 females (95% confidence intervals: 57–72).

In terms of population density (considering a 3-ha surface), estimates for females only varied between 23 and 27 individuals per ha assuming constant probability of capture or temporal variability of captures, but were 19 individuals per ha when a more realistic model incorporating both temporal and individual variability of capture probabilities are taken into account.

Population size estimates based on Jolly–Seber fluctuated remarkably across years, from less than 10 (2003) to about 40 (1994 and 2004, Table 1). Thus, female density ranged between 3.3 (2003) and about 13 individuals per ha (1994, 2004). Assuming a 1:1 sex-ratio, estimated density was 38.22±25.34 individuals per ha, with a range of 12–88. Survival estimates ranged from 0.25 to 1.286, being lowest in 2004 and highest in 2005 (Table 1).

Table 1. Results of a full Jolly–Seber open population model for gravid *H. viridiflavus* at the communal nesting site, years 1990–1997 and 2001–2009. Proportion of recaptures: estimated proportion of the population captured in each year from year 2; Marked animals in population: estimated number of marked animals in the population; Population estimate: estimated size of the population; Std. error pop. est.: standard error of the population estimate; Survival estimate: estimates of the proportion of the population surviving between years; Std. error survival: standard error of the survival estimates; New animals: estimate of the new animals entering the population, with negative numbers occurring when there has been a net loss of animals; Probability of capture: estimated probability of capture for each sample. Empty cells refer to cases when no values could be calculated.

Year	Proportion of recaptures	Marked animals in population	Population estimate	Std. error pop. est.	Survival estimate	Std. error survival	New animals	Probability of capture
1990		0			0.8	3.54E–05		
1991	0.67	4	6	0	0.67	0.27	8	1
1992	0.33	4	12	6.93	1.12	0.81	18	0.50
1993	0.29	9	31.5	26.54	0.61	0.45	23.4	0.22
1994	0.20	8.5	42.5	44.48	0.52	0.21	6.9	0.12
1995	0.43	6.5	15.2	6.71	1.14	0.74	6.7	0.46
1996	0.50	12	24	15.87	0	Not def.	0	0.25
1997	0.83	Not def.	Not def.	Not def.	Not def.	Not def.	Not def.	Not def.
2001	Not def.	Not def.	Not def.	Not def.	0.94	Not def.	Not def.	Not def.
2002	0.29	3.75	13.1	6.99	0.57	1.36E–05	0.8	0.53
2003	0.60	5	8.3	1.92	3.14	3.08	17.8	0.60
2004	0.50	22	44	43.36	0.25	Neg.	–3.5	0.09
2005	0.80	6	7.5	0.97	1.29	0.43	4.8	0.67
2006	0.62	9	14.4	4.72	0.67	0.24	6.4	0.56
2007	0.50	8	16	6.33	0.58	0.17	4.7	0.50
2008	0.50	7	14	3.90				0.71
2009	0.67							

CMR study

During the study period, we captured 48 adult snakes (28 males, 20 females; Appendix 2). Adult sex ratio did not depart significantly from 1:1 ($\chi^2=1.33$, df=1, $P=0.248$). The distribution of capture histories showed that, compared to females found at the communal nesting site, individual snakes of both sexes were captured for a significantly higher number of years (Mann–Whitney U-test: $Z=-2.564$, U=913.5, $P=0.0076$), with some individuals being recaptured in up to seven different years (Fig. 1B).

Total population size (including males and females), calculated in the assumption of a closed population model and a constant probability of capture among years, was 49±1.6 individuals (95% confidence intervals: 49–56). Population size was also within a range of 49–56 using the Chao estimator and the maximum likelihood Petersen–Schnabel census for temporal differences in capture probabilities, and was 59±7.61 (95% confidence intervals: 52–85) when the Chao individual variability in catchability was taken into account. A model with both individual and temporal change in capture probability incorporated gave an estimate of 50±1.6 adults (95% confidence intervals: 50–56). Population density varied, depending on the model used, between 16 and 18 individuals per ha; the most realistic model (with both temporal and individual variation in catchability taken into account) gave an estimate of 17 individuals per ha.

According to the Jolly–Seber model (Table 2), *H. viridiflavus* population size estimates fluctuated remarkably across years, ranging from about 14 (2003) to 26 (2005). Thus, the density of adult whip snakes ranged between 4.6 and 8.6 individuals per ha.

CNF versus CMR comparisons

Considering the various closed and open population models used, CNF population size estimates significantly exceeded those based on the CMR study (Wilcoxon test, $Z=2.023$, $P=0.043$). Considering only open population models, mean estimates for the whole study period were still higher based on CNF (38.2±25.3 individuals per ha) than based on CMR (22.7±5.3 individuals per ha). Nonetheless, comparing directly estimates for the years 2002–2009 (when both CNF and CMR sampling was done concurrently), there were no statistical differences between population sizes estimated with CNF and CMR (Mann–Whitney U-test: $Z=-1.143$, U=13, $P=0.253$). In the period 2002–2009, however, there was no relationships between the yearly population size estimate with

Table 2. Results of a full Jolly–Seber open population model for adult *H. viridiflavus* in the CMR study, years 2002–2009. For explanation of the columns, see Table 1. Empty cells refer to cases where no values could be calculated.

Year	Proportion of recaptures	Marked animals in population	Population estimate	Std. error pop. est.	Survival estimate	Std. error survival	New animals	Probability of capture
2002		0			0.75	2.64E–05		
2003	0.44	6.0	13.5	2.90	1.04	0.11	6.6	0.67
2004	0.55	11.5	20.7	2.26	0.72	0.07	11.9	0.87
2005	0.52	14.0	26.7	3.59	0.69	0.05	4.5	0.79
2006	0.72	16.6	22.9	2.03	0.88	0.09	3.5	0.78
2007	0.80	19.1	23.8	2.03	0.72	0.08	11.4	0.84
2008	0.58	16.4	28.4	4.20				0.67
2009	1							

CMR against CNF (Spearman's rank correlation coefficient = –0.257, *P*=0.623), showing that CNF samples did not capture the inter-annual variations in population sizes that CMR does. Overall, the magnitudes of differences in population size estimates between CNF and CMR were much higher with closed models than with open models (Fig. 2).

DISCUSSION

Our analyses provided contrasting evidence with regard to data obtained through CNF and CMR methods. On the one hand, CNF and CMR gave similar estimates of population size and density when the same subset in years for which field data are available was analysed with both methods (2002–2009). On the other hand, CNF provided population size and density estimates that were consistently higher than traditional CMR study when all years were included in the analysis (Fig. 2), particularly with closed population methods (poorly reliable in our scenario), and with a smaller difference when shorter time-spans were considered. More importantly, a weak correlation between CNF and CMR yearly counts revealed that CNF samples failed to capture the inter-annual variations in population sizes, thus making direct comparisons of the two methods very difficult. Based on this evidence, we conclude that CNF is not an entirely sound method for estimating population size in snakes, as it 1) tends to give higher estimates compared to more traditional CMR studies (although this was particularly evident with poorly reliable closed models), and 2) it does not allow the same inter-annual populations trends to be captured as by traditional CMR protocols. The latter problem may be particularly relevant, because population declines have usually been detected by long-term CMR studies (Reading et al., 2010). Although using traditional CMR studies to estimate population size and density is fraught with problems associated with the relative intractability of snakes (e.g. their elusiveness, temporal changes in density and potential for violating the assumption of equal catchability, e.g. Godley, 1980; Plummer, 1985; Luiselli, 2006), the considerable literature available on snake demography using the CMR approach may slightly mitigate these challenges (e.g. Plummer, 1985; Brown & Weatherhead, 1999; Altwegg et al., 2005).

It should also be mentioned that the differences in the estimates calculated from the 17-year CNF data and eight years of CMR data could be the result of actual differences in population sizes. Reading et al. (2010) recently published data on declines in snake populations, including a population of *H. viridiflavus* in France. Conversely, Reading et al. (2010) also published data showing a slight increase in another population of *H. viridiflavus* in France, thus suggesting that most snake populations undergo constant changes. If the Oriolo Romano population of *H. viridiflavus* underwent such changes in the nine years be-
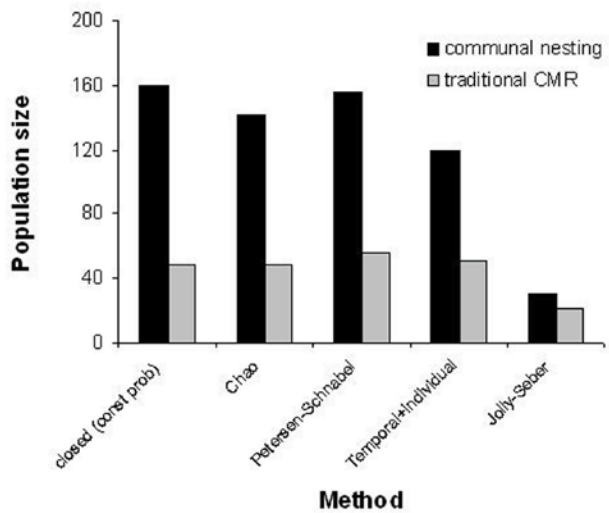

Fig. 2. Comparison of population size estimates for *H. viridiflavus* population at the study area based on counts of gravid females at the communal nesting site and on CMR of opportunistically collected individuals during random routes across the study area.

fore the CMR portion of this study began (an eventuality that could not be excluded *a priori*), this could result in real differences in population estimates, and perhaps in a more minor divergence between CNF and CMR estimates than suggested by the present analyses.

As already stated, our results based on closed models should be considered with caution. Indeed, although the CMR data would be less vulnerable to violations of model assumptions, it is still possible that, in a landscape with agricultural fields and intervening unmanaged vegetation as in our study area, snakes moving in and out of the study area can be a problem for detectability estimates. In fact, the inclusion of both snakes residing in the study area and those visiting it periodically may result in considerable individual capture heterogeneity (that even the zero-truncated Poisson test would be hard pressed to detect, especially with our small sample sizes), as well as potentially inflated density estimates. As it is impossible to know whether a given individual is resident in the study area or not, in our case the problem cannot be solved.

Besides the shortcomings of assuming close populations, we suspect that the reason behind the difference in population size estimates based on CNF and CMR is that, in order to reach their nesting site, several gravid females may travel distances beyond their usual home range. Dispersal to reach communal oviposition sites has also been documented in Swedish grass snakes, *N. natrix* (Madsen, 1984). As a consequence, female grass snakes had significantly larger home ranges than males (Madsen, 1984), which is uncommon in snakes (for *H. viridiflavus*, see Ciofi & Chelazzi, 1994; Lelièvre, 2010). The problem of an exaggerated population size estimate from samples of communal nesting females can be circumvented by enlarging the size of the area around the communal nesting site in which population density should be calculated. However, it is difficult to know precisely the maximum distance travelled by each female to reach the nesting site.

Although our study pointed out that using CNF samples for generating population sizes may be problematic, there are several reasons for conducting mark–recapture studies at communal egg-laying sites. For example, it is possible to explore whether female body size and/or condition affect probability of recapture or survival, whether seasonal fluctuations in prey affect female survival, or whether offspring marked near communal egg laying sites return to the same sites as adults. These (and other similar issues) may be appropriately explored using statistical programs such as MARK (White, 2006).

ACKNOWLEDGEMENTS

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APPENDIX 1

Gravid *Hierophis viridiflavus* captured during June 1990–1997 and 2001–2009

	1990	1991	1992	1993	1994	1995	1996	1997	2001	2002	2003	2004	2005	2006	2007	2008	2009
1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6		1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
7		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8			1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
9			1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10			1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
11			1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
12				1	0	0	0	0	0	0	0	0	0	0	0	0	0
13				1	0	0	0	0	0	0	0	0	0	0	0	0	0
14				1	0	1	0	1	0	0	0	0	0	0	0	0	0
15				1	0	0	0	0	0	0	0	0	0	0	0	0	0
16				1	0	0	0	0	0	0	0	0	0	0	0	0	0
17					1	0	0	0	0	0	0	0	0	0	0	0	0
18					1	0	1	1	0	0	0	0	0	0	0	0	0
19					1	0	1	0	0	0	0	0	0	0	0	0	0
20					1	0	0	0	0	0	0	0	0	0	0	0	0
21						1	1	0	0	0	0	0	0	0	0	0	0
22						1	0	1	0	0	0	0	0	0	0	0	0
23						1	0	1	0	0	0	0	0	0	0	0	0
24						1	0	0	0	0	0	0	0	0	0	0	0
25							1	1	0	0	0	0	0	0	0	0	0
26							1	0	0	0	0	0	0	0	0	0	0
27							1	0	0	0	0	0	0	0	0	0	0
28								1	0	0	0	0	0	0	0	0	0
29									1	1	0	1	0	0	0	0	0
30									1	0	1	0	1	1	0	0	0
31									1	1	0	0	0	0	0	0	0
32									1	0	0	0	0	0	0	0	0
33										1	0	0	1	1	1	0	1
34										1	1	0	1	1	0	0	0
35										1	0	0	0	0	0	0	0
36										1	0	0	0	0	0	0	0
37										1	1	0	1	0	1	1	1
38											1	1	0	0	0	1	0
39											1	0	0	1	0	0	0
40												1	0	0	0	0	0
41												1	0	0	0	0	0
42													1	1	1	0	0
43														1	0	0	0
44														1	1	0	0
45														1	0	1	0
46															1	1	1
47															1	1	1
48															1	0	0
49															1	0	0
50																1	0
51																1	1
52																1	1
53																1	0
54																1	0
55																	1
56																	1
57																	1

1 indicates captured in a given year.

APPENDIX 2

Male and female *H. viridiflavus* captured during spring 2002–2009 in a 3-ha area in Oriolo Romano.

Animal ID	Sex	2002	2003	2004	2005	2006	2007	2008	2009
1	Male	1	1	1	0	1	0	0	0
2	Male	1	0	1	1	1	1	0	0
3	Male	1	0	0	0	0	0	0	0
4	Male	1	1	1	0	0	0	0	0
5	Male		1	1	0	0	0	0	0
6	Male		1	0	1	1	1	1	0
7	Male		1	1	1	0	0	0	0
8	Male			1	1	1	1	0	1
9	Male			1	0	0	0	0	0
10	Male			1	1	1	0	0	0
11	Male			1	1	0	1	1	1
12	Male			1	0	0	1	1	1
13	Male				1	1	1	0	0
14	Male				1	0	0	0	0
15	Male				1	0	0	0	0
16	Male				1	1	1	1	1
17	Male				1	1	1	0	0
18	Male					1	1	1	1
19	Male					1	1	1	1
20	Male						1	0	1
21	Male						1	1	1
22	Male						1	0	0
23	Male							1	1
24	Male							1	1
25	Male							1	1
26	Male							1	0
27	Male							1	0
28	Female	1	1	1	1	0	0	0	0
29	Female	1	0	1	1	1	1	0	0
30	Female	1	0	0	0	0	0	0	0
31	Female	1	1	1	0	0	0	0	0
32	Female		1	1	1	1	0	0	0
33	Female		1	1	1	1	1	1	1
34	Female			1	0	0	0	0	0
35	Female			1	1	0	0	0	0
36	Female			1	0	0	0	0	0
37	Female				1	1	1	0	0
38	Female				1	0	1	0	1
39	Female				1	1	1	1	1
40	Female				1	0	0	0	0
41	Female				1	0	0	0	0
42	Female					1	1	0	0
43	Female					1	0	1	1
44	Female					1	0	0	1
45	Female						1	1	1
46	Female							1	0
47	Female							1	0
48	Female							1	1

1 indicates captured in a given year.