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Snakes and the Eternal City: variation in community metrics, body size and population density of snakes in contrasted landscapes of suburban Rome (Italy)

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Reports of global declines in animal populations are now numerous and also include snakes, a group of animals now widely regarded as bio-indicators. A prerequisite for any conservation management plan to protect or restore snake populations requires a data base that provides insight into population composition and changes. However, snakes are well known to be particularly difficult to quantitatively sample due to their secretive and elusive nature, and hence accumulating an adequate database for analysis requires long-term field studies that involve intensive searches. Populations of four snake species, Zamensis longissimus, Natrix helvetica, Vipera aspis and Hierophis viridiflavus living in two suburban areas of Rome with different extents of habitat alteration (deforestation), Vejo, a less altered site, and Tor Bella Monaca, a high altered site, have been monitored, but with interruptions since 1995. The results indicated that H. viridiflavus was the commonest species at both sites. Male bias was found in all four species but especially in Z. longissimus and V. aspis with detection of juveniles greatest in H. viridiflavus and N. helvetica. Snout to vent lengths (SVL) of H. viridiflavus and Z. longissimus, which were present at both sites, were greater at the less degraded habitat of the two study localities. Community metrics indicated that the degraded habitat had lower species richness, evenness, Shannon and Simpson diversity indices, but a higher dominance index. Recapture frequencies of snakes recaptured either once or multiple times were in general greater at Vejo. The highest population densities were found in H. viridiflavus, followed by V. aspis and N. helvetica, which were similar. However, long term trends in densities show declines in V. aspis and N. helvetica between 1995 and 2019. Population densities were in good agreement with density estimates found in previous studies of snakes in more natural habitats.

Keywords: snakes, long-term population changes, suburban areas, Rome, Italy

INTRODUCTION

Snakes are an important component of natural ecosystems regulating prey numbers such as rodents, or as prey species themselves (e.g. Greene, 2000). Recent reports of their and other reptiles' global decline are of major concern (e.g., Gibbons et al., 2000; Reading et al., 2010; Goiran & Shine, 2013), particularly as snakes have been defined as indicator species (Landres et al., 1988) for assessing the health of ecosystems. Indeed, research has suggested that predictions of snake extinction risk are underestimated (Böhm et al., 2013), but to understand the level of risk requires data on snake presence,

demography and densities in a variety of different habitats over long time periods (e.g., see Cayuela et al., 2019). Mark, release and recapture (MRR) is a wellknown and a frequently employed method of estimating animal numbers and often applied to snakes (e.g., Flatt et al., 1997; Bonnet et al., 2002; McDiarmid et al., 2011) but usually in natural and well-preserved environments (e.g., Webb et al., 2002; Stevenson et al., 2009; Cayuela et al., 2019). However, MRR presents logistical challenges since snakes are usually cryptic, secretive reptiles that are difficult to detect and identify (Kery, 2002; Willson et al., 2011), thus presenting considerable problems when attempting to accumulate an adequate data base for analysis (MacKenzie et al., 2002). Of interest and of

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particular value are snake densities in patches of suitable habitat within and around large cities (Burger et al., 2004; Pattishall & Cundall, 2008), where snakes may form small and isolated subpopulations with little genetic exchange between the various demes, and where inappropriate management of the "green areas" may compromise their survival (e.g. Vignoli et al., 2009; Bonnet et al., 2016). These patches of suitable habitat within large cities are arguably the most rapidly changing environments and the fragmented landscapes they produce have been cited as a major cause of loss of biodiversity (e.g. Fischer, 2000) by changing levels of predation risk (e.g. Evans, 2004), altering the thermal environment (Zhou et al., 2011) and reducing species abundance (Bateman et al., 2014). Therefore, it is reasonable to expect populations in these areas to experience the most rapid changes. However, few studies of reptiles have been carried out in these areas, especially in temperate regions (Capula et al., 1993; Rugiero & Luiselli, 2006; Luiselli & Rugiero, 2007; Simbula et al., 2019), including those of snakes (Tóth et al., 2002; Vignoli et al., 2009). Increasing our knowledge of snake population ecology in suburban areas is a key factor for effective management of their populations to enhance their survival probabilities.

The city of Rome, founded in the 8th century BC, has at present a population of around 4 million people (about 6 million if peripheral areas are included). The first major increase in population and expansion into surrounding areas that we know of began around 200BC and by the time of the first Emperor Augustus in 30BC Rome was the world's most populous city with an estimated one million or more inhabitants (e.g. Lo Cascio, 1994). The metropolitan expansion, after the end of the World War II was rapid with a resultant massive habitat loss and deforestation in the peripheral zones. This includes localities that once held dense snake populations, which were well known historically as indicated by the toponomastic (place name), for instance the "Serpentara" area because of the reported high snake densities. Rome, therefore, represents a prime region for examining snake presence and numbers in suburban areas with very few previous published studies (e.g. Vignoli et al., 2009). Additionally, recent research has shown that, in an area situated about 50 km north of Rome, changes in local climate (increases in annual temperatures) may be a factor in the decline of aspic viper (Vipera aspis) at hibernation dens (Luiselli et al., 2018), and it is therefore conceivable that suburban snake populations in the locality may have experienced similar declines. In this paper we present the first long term population study of snakes in urban environments in two areas of the city: La Storta (hereby VEJO) and Tor Bella Monaca (TBM). This information will not only increase our present understanding of suburban snake populations that were known during antiquity, but will serve as a data base to understand potential impacts of habitat alteration and climatic changes.

The main objectives of the study were:

 (i) to estimate species' presence, population densities, and general demographic aspects (sex ratio, body size, etc) of snakes in Rome's suburban areas with contrasted habitat characteristics (a well-preserved forestcultivation mosaic area versus a totally deforested and heavily degraded area).

(ii) to determine long-term population trends, i.e. identify any decreases in population densities as reported for other snake populations throughout the world (Reading et al., 2010).

Specifically, we investigate the following key questions:

(1) Are community metrics (diversity and dominance) different between the two study areas? We predicted that community evenness should be higher in the well-preserved habitat at VEJO but that species dominance should be higher in the degraded TBM habitat (Magurran, 1988).

(2) Are the body sizes of snakes greater in the wellpreserved VEJO habitat in comparison to the degraded habitat at TBM? We predicted that the average body size of snakes should be greater in the well-preserved environment due to greater abundance, diversity and density of prey species, particularly small mammals and saurians.

(3) Is the inter-annual variation in mean body size lower in the well-preserved habitat in comparison to the degraded habitat? We predicted that snake body size should be less prone to inter-annual oscillations in the higher quality habitat due to greater diversity of potential prey species. This should minimise the impact of any simultaneous collapse of prey populations in certain years.

(4) Are population densities declining with time greater in the degraded site than in the well-preserved site? We predicted that, because of heavy habitat loss, the snake populations in the degraded site may be more prone to declines than in the well-preserved site.

METHODS

Taxonomic note

The European herpetological fauna has undergone remarkable taxonomic changes during the last decade. Therefore, in this paper we followed the nomenclatural options as in Di Nicola et al. (2019).

Study areas

Surveys were carried out at two localities situated in the peripheral areas of Rome (Latium, central Italy): Tor Bella Monaca (TBM; 45 ha surveyed) on the north-eastern side of Rome and La Storta (VEJO; 55 ha surveyed), situated just north of the main ring road (Grande Raccordo Anulare). TBM is the altered/degraded site in the present study. It is a highly urbanised suburb in the eastern region of the city and the study site is primarily composed of two narrow strips of hedgerow (less than 10 m width and several hundreds meters length), bisecting wide agricultural land bordered mostly by light suburban areas (Fig. 1A). The primary habitat at VEJO is well preserved and composed of sections of oak woodland interspersed with agricultural land bordered by urban areas (Fig. 1B). Roads that interrupt habitat continuity bisect both VEJO and TBM. The list of potential prey species for snakes (small mammals, saurians and amphibians) at the two



Figure 1A. Google Earth images of the study site at Tor Bella Monaca with photographs illustrating the basic habitat structures.



Figure 1B. Google Earth images of the study site at Vejo with photographs illustrating the basic habitat structures.

study areas is given in Table 1 (bibliographic and personal data being pooled). The available data showed that VEJO has a considerably higher diversity and richness of prey species than TBM (19 versus 12 prey species).

Protocol in the field

In Mediterranean climates, it is frequently the case that snakes cannot be found during summer months (June-September) due to a combination of high temperature (>30° C) and prolonged periods of very low rainfall (Brito, 2003; Capula et al., 2014; Luiselli et al., personal observations) and so field-work was confined mostly to spring/early summer and autumn. Surveying varied between (CET) 0855 and 1745 hours. In total, 30601 minutes (510 hrs) were spent in the field searching for snakes at TBM and 34483 minutes (574.7 hrs) at VEJO, with a total of 3-4 field workers per season. Detection was through visual encounter by walking along both sides of the various hedgerows available at the study areas, in addition to walking within the cultivated land and the grasslands. When walking alongside the hedgerows, a distance of 2 m was maintained to minimise disturbance to encountered snakes. Snakes were individually marked by ventral scale-clipping and temporarily dorsally painted with a white number enabling the surveyors to identify individual snakes that were already marked, thus avoiding further pointless recaptures that may increase wariness in the snakes (Fig. 2). Each identified snake was sexed (by examination of the shape of the cloacal region and the tail), and measured for snout-vent-length (SVL), to ± 0.5 cm precision.

The results are derived from data using mark, release and recapture (MRR) commencing at VEJO during 1995, 1999, 2012 and 2019. At TBM, the MMR was carried



Figure 2. Examples of marked *H. viridiflavus* additionally painted with a white number on the back to minimise capturing of already marked individuals.

Table 1. List of the potential prey species available for snakes (small mammals, saurians and amphibians) recorded at the two study areas (bibliographic and personal data being pooled).

	Tor Bella Monaca	Vejo
Amphibians		
Bufo bufo	0	1
"green frogs"	0	1
Rana italica	0	1
Saurians		
Chalcides chalcides	1	1
Anguis veronensis	0	1
Podarcis muralis	0	1
Podarcis siculus	1	1
Lacerta bilineata	1	1
Tarentola mauritanica	1	1
Hemidactylus turcicus	1	1
Small mammals		
Suncus etruscus	1	1
Crocidura spp.	1	1
Talpa romana	1	1
Microtus savii	1	1
Myodes glareolus	0	1
Apodemus spp.	1	1
Rattus norvegicus	1	0
Rattus rattus	0	1
Mus musculus	1	1
Muscardinus avellanarius	0	1
TOTAL	12	19

out during 1998, 1999, 2000, 2012 and 2019. Logistical constraints impeded us to monitor the snake populations at the two study areas without temporal interruptions.

Statistical analyses.

Snake numbers at each site and recapture frequencies were evaluated using z-scores for differences between proportions p with the null hypothesis HO; p - p = 0 at α = 0.05. To compare snake abundance *G*-test goodness of fit tests were applied to the actual numbers caught and marked with the null hypothesis of equality of numbers the expected proportions at α = 0.05. Snout to vent lengths (SVL) were used to compare population parameters at different sites by ANOVA with post-hoc tests Tukey HSD for unequal sample sizes. This compares all possible pairs of mean values within and between sites with α = 0.05. Before analysis, normality tests were made using Anderson-Darling (a^2) for all data, male, female and pooled. This indicated most male and female data sets fitted a normal distribution (a^2 values from 0.17-0.54, P from 0.09 - 0.87) except in two of the four pooled data sets that were non–normally distributed, TBM 1998 (a^2 = 0.85, P = 0.02) and Vejo 2012 ($a^2 = 0.79$, P = 0.03). Hence, these data sets were Log, transformed prior to analysis.

Capture–mark–recapture analyses for estimating the abundance of the snake population at the two study areas were performed using methods for closed populations, with each sampling year treated as a distinct entity in the analyses (n = 4 years at VEJO and n = 5 years at TBM). 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. Considering the relatively long life of Mediterranean snakes (> 10 years; Fornasiero et al., 2016), we consider that temporal segments of 1 year are sufficiently short to justify the use of closed population models. In the analyses, we assumed constant natality and conflation of survival and emigration (sensu Rosewell & Shorrocks, 1987; Gilroy et al., 2012) for all snake species, at both study areas.

We used five different demographic models:

(1) 'Equal Catchability (M0)' (Pollock et al., 1990), or null model. This demographic model assumes that the probability of capture during the course of the study is the same for all individuals of the population.

(2) the Petersen–Schnabel method (Krebs, 1999). 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.

(3) 'Chao temporal change in capture probabilities (Mt)' (Chao, 1988). This demographic model assumes that the probability of capture of each individual is influenced by temporal parameters.

(4) 'Heterogeneity Model (Mh)' (Chao, 1988). In this demographic model, every individual of the sampled population has a different chance of being captured that is constant for all capture sessions (Pollock et al., 1990), that is determined by parameters such as sex and age.

(5) 'Both individual and temporal differences in capture probability (Mth)'. This demographic model assumes that the probability of capture varies depending on the temporal parameters and individual parameters (Chao et al., 1992).

Demographic analyses were carried out by Simply Tagging software (version 1.31; see Henderson & Seaby, 2002).

Diversity metrics of the snake assemblages between study areas were assessed by calculating various distinct univariate measures of community diversity for each site (Magurran, 1988):

1. Species richness; the total number of species recorded in each study area;

2. Dominance:

$$D = \frac{\sum_{i} n_i (n_i - 1)}{n(n-1)}$$

where *n_i* is the number of individuals of the taxon *i* and *n* is the total number of snakes that were recorded at each study area;

3. Simpson index: S = 1 - D, where diversity D lies between 1 and 0.

4. Shannon entropy index (Shannon & Weaver, 1963):



Figure 3. Histograms of total numbers of marked male, female and juvenile snakes found at both study localities.

$$H = -\sum_{i} \frac{n_i}{n} \ln \frac{n_i}{n}$$

where n_i is the number of individuals of each species at each site and n is the total number of snakes that were recorded at each study area.

5. Evenness, calculated by Buzan and Gibson's formula:

e[⊬]/S

with H' representing Shannon's index, and S the total number of snake species observed at each study area (Magurran, 1988).

6. Chao-1 index, indicating the theoretical number of species at each study area that can be expected on the basis of the sampling regime. The formula of the unbiased Chao-1 estimates is,

Chao-1 =
$$S + F_1 (F_1 - 1) / [2 (F_2 + 1)]$$

where F_1 is the number of singleton species and F_2 is the number of doubleton species at each study area.

Bootstrap analysis was applied to generate upper and lower confidence intervals of all indices based on 9,999 random samples, each with the same total number of individuals as in each original sample being generated (Harper, 1999). Diversity metrics analyses were carried out by Past 3.0 software. Apart from the demographic and diversity indices analyses, all other statistical tests were performed with Minitab (V17) software and use of various websites. All tests were two-tailed with alpha set at 5 %.

RESULTS

Sample sizes and community metrics

In total 298 snakes were captured and marked during the study period, 115 at TBM and 183 at VEJO. Three species



Figure 4. Diversity profiles **(a)** and saturation curves (with 95 % confidence intervals after 9,999 bootstraps) **(b)** for community diversity of snakes at the two study localities in suburban Rome.

were found and marked at TBM (*Hierophis viridiflavus* (n = 87), *Zamenis longissimus* (n = 28) with *Natrix helvetica* also recorded but not used in the analysis due to low sample size. At VEJO we found and marked *Vipera aspis* (n = 40), *H. viridiflavus* (n = 79), *Z. longissimus* (n = 23) and *N. helvetica* (n = 41). *Elaphe quatuorlineata* and *Coronella austriaca* were also found but not used due to low sample size. Therefore adequate sample sizes for analyses were only possible for *H. viridiflavus* and *Z. longissimus* at TBM, and *V. aspis*, *H. viridiflavus*, *Z.*

	Vejo	Lower	Upper	TBM	Lower	Upper
Species richness	6	5	6	3	2	3
Individuals	184	184	184	115	115	115
Dominance	0.288	0.2587	0.3325	0.6316	0.5636	0.7242
Simpson (1-D)	0.712	0.6674	0.7413	0.3684	0.2758	0.4364
Shannon (H)	1.394	1.284	1.473	0.5551	0.4482	0.6282
Evenness (e^H/S)	0.6715	0.6099	0.7611	0.871	0.7828	0.9371
Chao-1	6	5	7	3	2	3

Table 2. Diversity metrics for the snake communities at the two study areas. Upper = 95 % upper confidence limits; Lower = 95 % lower confidence limits. Confidence limits are calculated after 10,000 random Monte Carlo permutations



Figure 5. Boxplots of SVL's of marked snakes during different study periods at Tor Bella Monaca showing increases in SVL's of both *H. viridiflavus* and *Z. longissimus*. Top graphic shows *H. viridiflavus* and lower *Z. longissimus*. Triangles are mean SVL's, boxes represent the interquartile ranges with the ranges the vertical lines either side of the interquartile ranges.

longissimus and N. helvetica at VEJO.

Pooled data for all years indicated *H. viridiflavus* was the most abundant species at both sites; 76.6 % of the total at TBM (G = 32.5, p <0.0001) and 44.1 % of the total of the four species at VEJO (G = 36.1, p <0.0001). Among the species with adequate sample sizes, the least found was *Z. longissimus*, which formed 12.8 % of the samples at VEJO and 23.9 % at TBM.

Pooled data from all years was used to evaluate sex ratios (Fig. 3) and indicated males were consistently found in greater numbers than females in all species and at both sites. Closest to sex ratio equivalence was *N. helvetica* (1.17:1) and *H. viridiflavus* (1.44:1 at TBM & 1.12:1 at VEJO) with greater male bias in *V. aspis* (1.5:1) and especially *Z. longissimus* (1.75:1 TBM & 3.2:1 VEJO). As expected, due to greater difficulty in detection, juveniles were found in fewer numbers than either

males or females in all species and at both sites. Highest juvenile to adult ratios were found in *H. viridiflavus* (15 % at TBM and 8.9 % at VEJO) compared with juvenile *Z. longissimus* 4.3 % at TBM and 8.7 % at VEJO. However, generally adult/juvenile ratios were similar; at VEJO, juveniles formed 10.8 % of the *N. helvetica* sample and 7.3 % of *V. aspis*.

Analysis of community metrics indicated TBM had lower species richness, evenness, Shannon and Simpson diversity indices than VEJO, but a higher dominance index value (Table 2). Diversity profiles revealed that the VEJO snake community was significantly different from the TBM snake community (Fig. 4a). Saturation curves revealed that both communities were satisfactorily sampled throughout our field study (Fig. 4b).

Population SVLs

Adult snake SVLs at the two study areas, by year and by sex, are presented in Table 3 (TBM) and Table 4 (VEJO). Inter-site comparison of adult snake SVL was only possible in *H. viridiflavus* and *Z. longissimus*, which were present at both sites. The results indicated both species had greater SVL's at VEJO (ANOVA and Tukey post hoc; *H. viridiflavus* $F_{(9, 136)}$ =12.03, P<0.0001, *Z. longissimus* $F_{(8, 33)}$ = 4.5, P = 0.001).

Temporal variation in the mean SVL are given in Fig. 5 (TBM) and Fig. 6 (VEJO). At TBM, *H. viridiflavus* SVL increased by a total of 24.8 % and 11 % in *Z. longissimus*. Over the longer term, SVL's at VEJO were relatively stable between 1995 & 2019 in both *H. viridiflavus* (3.1 % decrease) and *Z. longissimus* (7.2 % decrease) with maximum differences of 8.2 % in *H. viridiflavus* (between 1995 and 2012) and 10.2 % in *Z. longissimus* (between a 1999 maximum SVL and 2019 minimum). In *V. aspis* there was an overall decline in SVL during the same sampling periods (mean SVL's from 67.5 to 58.9 cm; 12.7 % decrease) and in *N. helvetica* from 92.1 to 80.2 cm (12.9 % decrease).

Adult SVLs of *H. viridiflavus* were significantly different during the period of study at TBM ($F_{(4,69)} = 16.48$, P <0.0001). Tukey post hoc tests indicated SVLs during 2012 and 2019 were significantly greater than 1998, 1999 and 2000. SVLs of *Z. longissimus* also increased significantly during the same time period ($F_{(4,24)} = 3.74$, P = 0.02) with the groupings from the Tukey analysis indicating SVLs were significantly higher in 2012 and lower in 1999.



Figure 6. Boxplots of SVL's of marked snakes during different study periods at Vejo showing general SVL stability in all four species. Triangles are mean SVL's, boxes represent the interquartile ranges with the ranges the vertical lines either side of the interquartile ranges, horizontal lines are medians. Circles in the graph for *H. viridiflavus* are outliers. See text for further details.

Recapture frequencies and population size estimates

Recapture frequencies (Figs. 7 for TBM & 8 for VEJO) were in general greater at VEJO (66.3 ± 8.5 %; 4 species) and highest in *V. aspis* (75 %) and *N. helvetica* (73 %). At TBM mean recapture rate was 25 ± 12 % (2 species) with *H. viridiflavus* more frequently recaptured than *Z. longissimus* (37 % versus 13 %; *z* = 3.09, P = 0.002; Fig. 7). Comparisons of pooled recapture data from both sites indicated the differences between VEJO and TBM were significant (*z* = 5.8, P <0.001). Intra-specific comparisons indicated multiple recaptures (snakes that were recaptured more than one time) were also higher at VEJO in both *H. viridiflavus* (32 versus 12.5 %; 2 – tailed P, *z* = 3.1, P = 0.002) and *Z. longissimus* (38.1 versus 3.3.1 %; *z* = 3.06, P = 0.001; Fig. 8).

Short-term analysis at TBM.

In 3 consecutive years of sampling at TBM (1998, 1999 & 2000) initially 8 males, 4 females and 3 juvenile H. viridiflavus were marked during 1998 (111.8 hours searching). In 1999, 4 of these (3 males, 1 female (27 %) were recaptured along with 10 previously unmarked snakes (3 males, 5 females, 2 juveniles; 101.65 hours search). Of 25 snakes now marked (1998+1999), none were recaptured during 2000 (73.1 hours search effort) but 12 (6 males, 5 females, 1 juvenile) new individuals were captured. During the same time period (and hours of search effort) at TBM, 4 (3 males, 1 female) Z. longissimus were marked in 1998. Two of the males were recaptured in 1999 along with 6 new captures (3 males, 2 females, 1 juvenile). None of the marked snakes from 1998/1999 were found in 2000 although 5 (2 males, 3 females) new snakes were found along with one deceased individual. Recaptures at TBM showed similar within-year recapture rates for male (38.1 %) and female (33.3 %) *H. viridiflavus* (z = 0.293, p = 0.38). Female *Z. longissimus* were recaptured more frequently than males (33 versus 20 %) but the sample size was small and the result not significant (z = 0.60, p = 0.27).

Population density estimates.

Tables 5A & B show the full results for the demographic models applied to snake captures and recaptures in the two study localities; Figure 9 shows graphical summaries. Population density and standard error estimates from the 5 models were in remarkably good agreement at VEJO. In general, the highest densities were for H. viridiflavus (mean±std dev of 5 model estimates: 25.4±9.7 snakes in the 55 ha study locality). Numbers of V. aspis and N. helvetica were similar, 12.1±4.9 and 12.1±4.8 respectively. Lowest estimates were found in Z. longissimus (8.1±0.7). However, we believe this may in part be due to the very low capture rates in this species due to its more secretive behaviour, which rendered 1999 data insufficient to make a robust analysis. The trends in densities showed a 65 % reduction in V. aspis and 73 % in N. helvetica between the 1995 and 2019. Smaller declines were found in Z. longissimus (12.2 %) and H. viridiflavus (5.4 %) but these are what might be expected under natural population fluctuation.

The TBM (45ha) estimates showed less agreement between models with error rates on combined estimated mean values varying from 2.5 to 30.6 % and incomplete in three of 5-year estimates for *Z. longissimus*. Again, data for the latter species is probably due to low initial number of marked snakes and especially recaptures. The estimates for *H. viridiflavus* varied between years with mean estimates of the five models ranging from 6.2 snakes in 2012 to 29.4 in 2000. We could find no evidence of declines in density in *H. viridiflavus* at TBM.

Table 3.	Population parameters of marked adult snakes (juveniles omitted) at Tor Bella Monaca given as snout to vent lengths
(SVL) in	cm. Statistical tests are one-way ANOVAs for comparisons of male versus female size differences (where possible).
Values w	<i>v</i> ith * attached indicate a single specimen.

Year	Species	Sex	Mean	Std Dev	Max	Min	n	F	Р
1998	H. viridiflavus	males	79.5	9.2	90.8	70.5	8		
1998	H. viridiflavus	females	72.3	5.9	79.3	64.8	4	2.04	0.18
1999	H. viridiflavus	males	83.4	10.2	98.0	90.5	11		
1999	H. viridiflavus	females	78.5	8.0	98.5	64.8	8	1.29	0.27
2000	H. viridiflavus	males	92.5	15.2	108.5	74.0	6		
2000	H. viridiflavus	females	80.6	7.4	89.0	72.5	5	2.25	0.15
2012	H. viridiflavus	males	102.4	10.3	113.0	88.4	7		
2012	H. viridiflavus	females	95.8	11.2	111.3	79.6	6	1.23	0.29
2019	H. viridiflavus	males	105.2	10.5	119.3	92.8	10		
2019	H. viridiflavus	females	96.9	9.3	109.1	87.4	7	2.28	0.11
1998	Z. longissimus	males	100.9	4.3	105.0	96.5	3		
1998	Z. longissimus	females	91*	NA	NA	NA	1	NA	NA
1999	Z. longissimus	males	100.3	6.8	107.5	88.7	6		
1999	Z. longissimus	female	92.4	1.4	93.8	91.0	3	3.7	0.1
2000	Z. longissimus	male	99.3	10.2	116.5	90.0	5		
2012	Z. longissimus	male	114.4	12.5	126.2	97.6	4		
2012	Z. longissimus	female	106.5	2.9	108.6	104.5	2	0.69	0.45
2019	Z. longissimus	male	108.3	8.1	117.4	102.0	3		
2019	Z. longissimus	female	112.2*	NA	NA	NA	1	NA	NA

Table 4. Population parameters of marked adult snakes (juveniles omitted) at Vejo given as snout to vent lengths (SVL) in cm. Statistical tests are one-way ANOVAs for comparisons of male versus female size differences (where possible). Values with * attached indicate single or no specimens, p values in grey indicate significant results.

Year	Species	Sex	Mean	Std Dev	Max	Min	n	F	Р
1995	H. viridiflavus	males	107.4	6.7	117.0	94	13		
1995	H. viridiflavus	females	100.7	2.9	103.6	96.0	7	6.02	0.02
1999	H. viridiflavus	males	108.4	7.5	121.5	98.0	10		
1999	H. viridiflavus	females	101.4	8.0	112.4	79.4	15	4.87	0.04
2012	H. viridiflavus	males	101.1	9.4	115.8	91.6	6		
2012	H. viridiflavus	females	96.9	4.2	103.2	90.7	6	1.0	0.34
2019	H. viridiflavus	males	106.2	7.3	116.0	91.7	7		
2019	H. viridiflavus	females	100.3	3.0	104.8	97.4	7	2.62	0.13
1995	Z. longissimus	male	119.7	10.1	129.4	106.0	4		
1995	Z. longissimus	female	103.7	9.8	109.6	89.0	4	5.32	0.06
1999	Z. longissimus	male	121.7	13.7	131.4	112.0	2		
1999	Z. longissimus	female	NA	NA	NA	NA	NA	NA	NA
2012	Z. longissimus	male	123.3	4.0	127.4	119.5	3		
2012	Z. longissimus	female	112.3	8.3	118.2	106.5	2	4.3	0.13
2019	Z. longissimus	male	115.1	10.6	128.4	102.2	5		
2019	Z. longissimus	female	111.4*	NA	NA	NA	1	NA	NA
1995	N. helvetica	male	80.8	8.1	81.5	69.6	9		
1995	N. helvetica	female	101.2	9.0	121.4	92.2	8	24.25	<0.0001
1999	N. helvetica	male	82.5	6.0	88.0	75.0	6		
1999	N. helvetica	female	105.3	10.7	117.0	96.0	3	17.72	0.004
2012	N. helvetica	male	75.2	7.1	81.0	67.3	3		
2012	N. helvetica	female	100.3	8.8	106.6	94.1	2	12.8	0.04
2019	N. helvetica	male	66.9	2.3	68.5	64.3	2		
2019	N. helvetica	female	93.4	0.21	93.6	93.3	2	272.9	0.004
1995	V. aspis	male	65.3	5.4	71.3	58.5	6		
1995	V. aspis	female	61.8	3.7	65.0	56.9	4	1.23	0.30
1999	V. aspis	male	65.9	5.7	73.2	58.7	5		
1999	V. aspis	female	62.1	2.1	65.0	59.6	5	2.0	0.19
2012	V. aspis	male	61.6	7.4	73.1	55.5	5		
2012	V. aspis	female	63.7	0.2	63.8	63.1	2	0.14	0.72
2019	V. aspis	male	58.2	13.8	68.1	35.6	5		
2019	V. aspis	female	60.6	0.5	61.0	60.3	2	0.06	0.82



Figure 7. Recapture frequencies at Tor Bella Monaca for *H. viridiflavus* and *Z. longissimus*. Cells shown in black in all graphs represent snakes that were caught, marked and released, grey cells those that were recaptured once and cross hatched cells snakes captured more than once in a single season. See text for further details.



Figure 8. Recapture frequencies at Vejo for *H. viridiflavus, Z. longissimus, N. helvetica* and *V. aspis*. Cell markings are those in Fig 7. See text for further details.

The incomplete estimates for *Z. longissimus* prevented an evaluation.

DISCUSSION

General considerations

The results of this study revealed an imbalance of species presence, size (SVL), recapture frequencies and population densities between the two study areas. However, in general, we observed two similarities between the study sites: a male-skewed adult sex ratio and a relative scarcity of captured juveniles. The bias in males in all four species is not unusual in snake populations (Parker & Plummer, 1987) and may result from greater female mortality and the costs of reproduction (e.g., Madsen & Shine, 1993; Luiselli et al., 1996; Shine, 2003). Absence of juveniles in the sampling is typical of snake field studies (Pike et al., 2008; Sewel et al., 2016; Cayuela

et al., 2019) and unfortunately this limits knowledge of juvenile sex ratios and other aspects of their biology (Pike et al., 2008). Additionally, the absence of any recaptures during 2000 in the short-term analysis at TBM illustrates the difficulties separating dispersal and mortality in MRR studies. For example, age estimates of H. viridiflavus show relatively long live spans potentially reaching 20+ years (Fornasiero et al., 2016), which suggests that at least some 1998/1999 marked snakes were likely present but not found. A possibility is that three of the species in the present study H. viridiflavus, Z. longissimus and N. helvetica are wide foragers moving extensively over the landscape, which may reduce frequency of re-sighting during field searches. Sewell et al., (2016) indicated that very high population estimates for *N. helvetica* in England was possibly due to temporal residence of some individuals in a patch of high-quality habitat within a larger home range. Furthermore Gregory (2013)

Table 5. Results for the various demographic models applied to snake captures and recaptures in the two study areas by year. (A) Tor Bella Monaca; (B) Vejo. Population density estimates given as means and standard deviations (±) derived from the 5 population models at Tor Bella Monaca. Maximum-minimum values are the range of standard errors on each of the model means generated by the 5 models. Number of marked snakes (n) in each sampling period is also shown. Low sample size and recaptures in *Z. longissimus* negated analysis for 1998, 2012 and 2019 at TBM (A) and during 1999 at Vejo.

A) Tor Bella Monaca (TBM) 45 ha study area

Year	Species	Population Size (mean)	±	Max- min ±	n
1998	H. viridiflavus	15.8	0.4	1.094-1.846	15
1999	H. viridiflavus	20.4	4.0	0- 5.958	25
2000	H. viridiflavus	29.4	9.0	1.797-13.42	12
2012	H. viridiflavus	6.2	0.4	0.487-1.115	13
2019	H. viridiflavus	22.8	1.7	1.09-2.503	21
1998	Z. longissimus	NA	NA	NA	4
1999	Z. longissimus	14.8	3.5	1.399-10.17	8
2000	Z. longissimus	18.0	1.1	0.689-1.448	5
2012	Z. longissimus	NA	NA	NA	6
2019	Z. longissimus	NA	NA	NA	4

B) Vejo 55 ha study area

Year	Species	Population Size (mean)	±	Max- min ±	n
1995	H. viridiflavus	20.3	0.43	0.055-1.305	23
1999	H. viridiflavus	42.2	2.4	5.589-8.169	29
2012	H. viridiflavus	20.0	6.29	1.106-16.49	13
2019	H. viridiflavus	19.2	1.72	1.159-4.84	16
1995	V. aspis	20	0	0.008 - 0.396	12
1999	V. aspis	11.8	0.74	0.111-2.646	11
2012	V. aspis	9.4	4.32	0.001-0.408	8
2019	V. aspis	7	0	0.0-0.551	7
1995	Z. longissimus	8.2	0.4	0.008-1.323	8
1999	Z. longissimus	NA	NA	NA	2
2012	Z. longissimus	9	1.54	1.268-5.292	6
2019	Z. longissimus	7.2	0.4	0.593-1.305	7
1995	N. helvetica	18.5	0.5	0.019 - 1.871	19
1999	N. helvetica	13.2	1.17	1.178 -3.658	11
2012	N. helvetica	11.6	2.33	1.308-7.552	6
2019	N. helvetica	5	0	0-0.936	5

observed increased wariness in *N. helvetica* after first detection compared to first-time captures. It is therefore possible that repeated capture could result in some individual snakes moving elsewhere suggesting that the use of a secondary mark to enable identification at a distance, as employed here, has value in reducing this effect. These results illustrate just some of the difficulties in calculating snake population densities, that sampling area and home ranges of certain species may not be synonymous and highlight the value of long term MRR studies in understanding snake population ecology.



Figure 9. Histograms of estimated population densities of snakes at both 45ha and 55ha sites during the various study periods. More detailed information is given in the text and in Table 5.

Were community metrics (diversity and dominance) different between the two study areas?

Our prediction that community evenness should be higher in the well-preserved area (VEJO) and species dominance in the degraded site (TBM) was confirmed. This supports the notion that viable snake populations likely require the protection of large, contiguous sections of unfragmented habitat (Dodd & Barichivich, 2007), with some Mediterranean species at least particularly affected by habitat fragmentation processes (Luiselli & Capizzi, 1997). Indeed high quality habitat was suggested as one of the key factors involved in very high densities of *N. helvetica* in England (Sewell et al., 2016). The dominant/most abundant species at both our sites, in terms of number of individuals and probably also biomass was H. viridiflavus, a wide-ranging forager with a relatively broad-based diet including mammals, birds, reptiles and amphibians (Capizzi et al., 2008; Lelièvre et al., 2012). These dietary characteristics likely explain H. viridiflavus greater abundance, which has been found in other snakes with a similar lifestyle (e.g. Fitch, 1999). A wide foraging lifestyle increases contact with prey species and could enhance adaptation to changing environments compared to a sentinel (ambush-foraging) lifestyle (e.g. V. aspis). In general, H. viridiflavus is almost invariably the most abundant snake species in terrestrial Mediterranean environments in Italy and the least affected by habitat fragmentation processes (Luiselli & Capizzi, 1997 although see Storniolo et al., 2019). Thus, our data in this regard support the findings of earlier studies.

Were body sizes of snakes greater in the well-preserved site than in the degraded site?

Two species, *H. viridiflavus* and *Z. longissimus*, were present in both study localities but SVL's were significantly greater in the higher quality habitat at VEJO where a much higher diversity of potential prey species was observed. This confirms our a-priori prediction. Unfortunately, there are no data in the literature concerning the variation of the body sizes of snakes in relation to habitat fragmentation for any meaningful comparison. However, there is evidence that maximum body size co-varies with prey density in squamate reptiles (e.g. Jessop et al., 2006).

Was the inter-annual variation in mean body size lower in the well-preserved area than in the altered area?

The body sizes of all species were relatively stable across years at VEJO, but snake numbers fluctuated and showed increased size in the latter years in the two species at TBM. This confirms Hypothesis 3. The diet composition of H. viridiflavus and Z. longissimus is relatively similar and includes small mammals (Capizzi et al., 2008; Lelièvre et al., 2012). It is possible that increases in body size were driven by increases in small mammal density at TBM during recent years as a consequence of changes in waste management in the area, for example increases in rat numbers due to inadequate waste disposal (Traweger et al., 2006). In addition, higher numbers of rats may have impact on smaller-sized mammal numbers (Capizzi & Santini, 2007; Harris & McDonald, 2007). These factors could favour selection of larger snake body size due to the need to forage upon and subdue, larger prey.

Were population densities declines with time greater in the altered site than in the well-preserved site?

Our data here gave conflicting results indicating relatively stable densities in *H. viridiflavus* and *Z. longissimus* at both sites, thus contradicting our a-priori prediction. The slight downward shift in numbers in the former we tentatively attribute to natural population fluctuations but it is worth noting that declines in *H. viridiflavus* numbers have recently been reported in central Italy (Storniolo et al., 2019).

However, notable declines were observed in population densities of both *V. aspis* and *N. helvetica* at VEJO, although the underlying causes of these apparent declines are not immediately obvious. Rome has experienced annual temperature increases between 1999 and 2011 of approximately around 3° C (Capula et al., 2016). The temperature increases associated with long term phenology and population decline of *V. aspis* north of Rome (Luiselli et al., 2018) could similarly be impacting on populations in the city by influencing offspring fitness (Bonnet et al., 2002) and reproductive effort. This is especially likely in *V. aspis*, a typical capital breeder that delays reproduction until large energy reserves have been amassed resulting in females producing only a single litter during their lifetime (e.g. Bonnet et al., 1999; Bonnet et al., 2002). In theory, a prolonged season should allow for increased feeding, and thus energy uptake for reproduction (Rugiero et al., 2013) but increased temperatures and subsequent shorter hibernation periods may increase metabolic costs as a consequence of shorter hibernation periods delaying energy uptake and reproduction. A direct consequence of this is a requirement for females to survive for longer periods before reproduction can occur which increases exposure to predation risk before reproduction can take place. Additionally, feeding opportunity relates to prey density, which depends on habitat quality and climate, in particular rain for increased plant growth, and populations of mice/invertebrates, lizards/snakes (Rugiero et al., 2012). However, a warmer climate does not guarantee increased rainfall and resources; there was no correlation between rainfall patterns and climate warming in the Rome area throughout the study period (Rugiero et al., 2012, 2013).

Thermal influences on offspring fitness and survivorship have also been found in N. helvetica in northern Europe where optimum temperatures during embryonic development result in larger fitter offspring capable of enhanced locomotor performance (Löwenborg et al., 2011; Hagman et al., 2012). Deviations from optimum temperatures during embryonic development have been associated with low survivorship in adults (Löwenborg et al., 2012). There is also evidence that in natural environments female N. helvetica communally select nests with optimum thermal properties in good agreement with the findings of laboratory studies (e.g. Löwenborg et al., 2011; Hagman et al., 2012; Meek, 2017). To access nesting areas involves travelling across the landscape (Meek, 2017) which increases mortality risk (Bonnet et al., 1999) suggesting that the thermal benefits derived from migrating to nesting areas outweigh the risk of movement. The key role temperature plays in snake ecology therefore cannot be underestimated and it is perfectly feasible that global warming may be impacting on snake numbers in many ways, even at the higher quality habitat at VEJO as a consequence of impacts on their thermal ecology. Local selection should favour individuals that are better at selecting the best possible nesting sites, especially across generations, and it cannot be excluded that snakes from VEJO and TBM may be constrained to adjust behaviour similar to that found in more southern conspecific populations that are experiencing greater climate warming pressures.

Population densities (number of individuals per ha) of all species averaged from 0.3 to 0.7 individuals per ha per year. The low density observed at the two study areas may possibly be related to low prey and shelter (predation cover, oviposition, hibernation) densities, as a consequence of reduced microhabitat complexities. Nonetheless, our density estimates are in good agreement with estimates for conspecifics reported in earlier studies (e.g., see Storniolo et al., 2019, and references therein).

At a local level, the densities of snakes in suburban areas of Rome are not generally lower than in more natural areas, and are able to persist if habitats are suitable with abundant food resources. Nevertheless,

when populations are < 30 individuals there may be a risk of inbreeding, genetic drift or population collapse through increased mortality in certain years (by severe winters, drought, low prey density, disease etc., Madsen & Shine, 1992; Madsen et al., 1996; Guicking et al., 2004). It is of interest and surprising that such populations have persisted for over two decades (and much longer) without any apparent immigration. However, we cannot be certain of the latter despite our inability to detect immigration at either localities but it is possible that some individuals may have immigrated into the localities by way of hedgerows bordering the extended agricultural lands surrounding the study areas (especially at VEJO). This is potentially most likely to be found in the wide foraging H. viridiflavus, Z. longissimus and N. helvetica but also in V. aspis, which although an ambush-foraging predator, is known to move to and from spring and summer home ranges using hedgerows as ecological pathways (Saint Girons, 1996). Therefore, as a conservation management practice, it is imperative that the network of hedgerows is preserved, indeed increased, in order to conserve the historic remnant isolated snake populations that inhabit the suburban areas of Rome and the Mediterranean region as a whole.

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