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A long-term study of a snake community in north-western Tuscany (central Italy): population structure and density patterns

Eine Langzeit-Studie über eine Schlangengemeinschaft in der nordwestlichen Toskana (Mittelitalien): Populationsstruktur und Dichtemuster

Federico Storniolo[1], Sacha Menichelli[1], Marco A.L. Zuffi[1]

1 *Museum of Natural History, University of Pisa, via Roma 79, 56011 Calci (PI)-Italy*

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Corresponding author: *Marco A.L. Zuffi* (marco.zuffi@unipi.it)

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Abstract

We analysed a snake species community of a Mediterranean 0.2 ha ecotonal area during a 21 year time span, monitoring two colubrid and one viperid snake species. We carried out analyses in seven years (1997, 1999, 2002, 2004–2005, 2016–2017) that had similar sampling efforts and, in the last two years of short-term monitoring, we applied a recently proposed monitoring protocol of the Italian Environment Ministry. In total, we captured 172 distinct individuals, 61 whip snakes (*Hierophis viridiflavus*), 26 barred grass snakes (*Natrix helvetica*) and 85 asp vipers (*Vipera aspis*). Regarding the long-term monitoring period, whip snakes were captured on average about nine times per year, grass snakes were captured four times per year and asp vipers were found 12 times per year. Captures decreased in whip snakes, while increased in grass snakes and remained constant in asp vipers. In 2016 and 2017, we captured 10 whip snakes, 19 grass snakes and 31 asp vipers. Density estimates of snake species (0.5 *H. viridiflavus*/ha, 0.3 *N. helvetica*/ha and 0.7 *V. aspis*/ha) differ, to a certain extent, from published results for some other areas of central, northern and western Europe, perhaps depending on the approach applied for habitat suitability estimation. The average body size between two years differed neither for whip snakes nor for grass snakes, but it decreased significantly in asp vipers.

Kurzfassung

Wir untersuchten eine aus zwei Natternarten und einer Vipernart bestehende Schlangengemeinschaft eines mediterranen Ökotons über einen Zeitraum von 21 Jahren. Wir analysieren 7 Jahre (1997, 1999, 2002, 2004–2005, 2016–2017) mit ähnlicher Sammelintensität; für die letzten beiden Jahre brachten wir mit engmaschiger Überwachung ein kürzlich vorgeschlagenes Monitoring-Protokoll des italienischen Umweltministeriums zur Anwendung. Insgesamt fingen wir 172 Individuen, davon 61 Gelbgrüne Zornnattern (*Hierophis viridiflavus*), 26 Barren-Ringelnattern (*Natrix helvetica*) und 85 Aspisvipern (*Vipera aspis*). Auf den langfristigen Untersuchungszeitraum bezogen wurden durchschnittlich 9 Gelbgrüne Zornnattern, 4 Ringelnattern und 12 Aspisvipern pro Jahr gefangen. Die Fangquote für Gelbgrüne Zornnattern nahm ab, während sie für Barren-Ringelnattern anstieg und für Aspisvipern konstant blieb. In den Jahren 2016 und 2017 fingen wir 10 Zornnattern, 19 Ringelnattern und 31 Aspisvipern. Die Dichten der Schlangenarten (0.5 *H. viridiflavus*/ha, 0.3 *N. helvetica*/ha and 0.7 *V. aspis*/ha) unterscheiden sich etwas von Literaturangaben für andere Gebiete von Mittel-, Nord- und Westeuropa, vielleicht abhängig von der angewandten Erfassungsmethode zur Abschätzung der Habitateignung. Die durchschnittliche Körpergröße zwischen zwei Jahren unterschied sich weder für Zornnattern noch für Ringelnattern, aber nahm für Aspisvipern signifikant ab.

PENSOFT.

Key Words

central Mediterranean Italy, Colubridae, density, long-term analysis, Reptilia: Serpentes, Squamata: species occurrence, Viperidae

Introduction

Although modern biological and zoological research have shown the importance of snakes in environmental studies as *indicator species* (Landres et al. 1988) in terms of habitat use, activity patterns, reproduction and diet (Scali and Montonati 2000; Rugiero et al. 2002; Filippi et al. 2005; Capula et al. 2006; Row and Blouin-Demers 2006), relatively few studies have been performed for long-term analyses (see Reading et al. 2010 for comprehensive review and discussion). Most recent studies focused only on one single species as an indicator for ecosystem assessment (Thomas 1982; Soulé 1991; Lindenmayer et al. 2000): after checking on Web of Science for "snakes AND ecology" for instance, we found 374 published papers since 1971. Randomly checking about 50% of them and excluding those connected with review papers, we found only 23 papers concerned with long-term surveys and analyses (> 4 yrs). Specifically, a smaller number of papers considered longer time spans, as > 4 yrs and < 10 yrs (Gentilli et al. 2006), > 10 yrs (Keogh et al. 2007; Bonnet et al. 2011; Miller et al. 2011; Leliévre et al. 2013) and those embracing 15 or even more years were very few (Jäggi and Baur 1999; Sullivan 2000; Moreno-Rueda and Pleguezuelos 2007; Pringle et al. 2009; Rugiero et al. 2013; Capula et al. 2014). Over a much longer period, population decline has been assessed in *Drymarchon couperi* (Godley and Moler 2013) and long term snake community dynamics have been analysed by Fitch (1999) in North America, during a 50 year period. As ectotherms, snakes are often specialised in surviving in low energy environments and are highly adaptive and plastic to environmental changes and food availability reduction, as during reproduction (Shine 1979), in comparison with terrestrial endotherms (Martin 1987). In addition, snakes also show high longevity (Fornasiero et al. 2016) and this feature makes them effectively suitable for long-term trend analyses on an ecosystem-scale, for instance in community or population changes (Beaupre and Duvall 1988).

Furthermore, given their intrinsic characteristics as wide home ranges of some species (Ciofi and Chelazzi 1991), disruptive dorsal pattern (Jackson et al. 1976), elusive and shy behaviour (Bishop and Gendron 1998), snakes are undoubtedly organisms difficult for study.

Recently, the European Union has underlined the need for continuous monitoring plans of animal and plant species and habitats of community interest, aimed at environmental assessment, conservation, protection and management of the species and habitats (Council Habitats/Directive 92/43/EEC of 1992: article 17). According to this point, monitoring protocols have been adopted in Italy in the last years (Italy: Stoch and Genovesi 2016). The Italian Ministry established standard procedures and rules to obtain valuable and suitable data on all plant and animal taxa in the Habitats Directive, in the frame of the National and European Strategy for Biodiversity (Stoch and Genovesi 2016).

We have been therefore aimed at considering i) population dynamics and fluctuations of a snake community through a long time span (Sullivan 2000; Pringle et al. 2009) to evaluate what, if any, fluctuations the studied community has undergone and, if possible, to correlate such fluctuations to changes in biotic/abiotic environmental factors; ii) the performance of a short term analysis by applying the census and monitoring techniques for snakes as in the ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) and Italian Ministry rules (Mezzasalma et al. 2016; Stoch and Genovesi 2016), in order to assess if and how protocols are valuable, repeatable and sharable tools amongst specialists.

Long and short term analyses are intended to provide suitable information about species relative abundance and habitat suitability.

Materials and methods

The studied species

In this research, carried out in Tuscany, Central Italy, we considered four species of snakes that are commonly widespread throughout the Italian territory. The Atlas of amphibians and reptiles of Tuscany (Vanni and Nistri 2006) reports eight species of snakes in the whole region and four are expected in our sampling site according to the Atlas and excluding any human introduction: three colubrids [*Hierophis viridiflavus*, the European Whip snake, *Natrix natrix*, the grass snake (now *N. helvetica* as in Kindler et al. 2017) and *Zamenis longissimus*, the Esculapian snake] and one viperid (*Vipera aspis*, the asp viper). Only the European whip snake (*H. viridiflavus*) and the Aesculapian snake (*Z. longissimus*) are included in the Habitats Directive, but we have also applied the monitoring protocol to the other two species.

Study area and sampling procedures

The studied area is an ecotone characterised by the transition from the Tombolo mixed forest (Pisa, central Italy) and the Tyrrhenian coast (43°38'52.63"N, 10°18'18.28"E) (DMS), next to the Arnino Laboratory (Department of Biology, University of Pisa), formerly a cultivated field and now abandoned. The ecotone is north-south orientated (facing westwards), with a small pathway dividing the area in two contiguous segments of 500 m length each, for a total of 1000 m, 2 m width and an overall estimated surface area of 0.2 ha. It falls inside the range of the SIC ("Sito di Importanza Comunitaria"), now ZSC ("Zona Speciale di Conservazione") "Selva Pisana" (code IT5170002), an area of the Regional Park "Migliarino San Rossore Massaciuccoli".

Sampling and data recording were carried out from 1995 to 2017 with different sampling efforts. Continuous and comparable research efforts, during the whole of the snake's active season, were carried out in 1997, 1999, 2002, 2004–2005, 2016–2017: sampling has been carried out from the beginning of the snake's outdoor activity to the end of active season (March-October), on average twice a week by two or three operators (one being the same during the whole study period, MALZ) for 5 hrs per sampling session. We performed the sampling by repeated surveys along the ecotonal transect, alternating North to South and vice versa, patrolling the direction and ensuring that the sampled area was covered during all day hours. Snakes were visually detected, captured by hand and carefully examined, sexed, measured and marked for further recognition, with ventral clipping and a serial enumeration system, suitable for at least four years (Brown and Parker 1976). They were released at the capture point after data recording.

The long term analysis considered all different snake age classes, we recorded (newborns, juveniles, adult males, adult females) and we aimed at verifying general life-history dynamics of each species, that is population stability, decrease or increase.

The short term analysis was carried out with the aim of controlling the area of previous research, after ten years of no research, in order to verify any change in occurrence patterns and species abundance. In parallel, the monitoring protocol at national level (Mezzasalma et al. 2016) provides that transects should be four per area, repeated at least six times per site within a six year span. Such a protocol provides confirmation of the presence of snake species, when observations gave positive results during consecutive surveys. Therefore, we have tested the monitoring protocol procedure and suitability on our target ecotone, even if it represents only one site. Therefore, we have also estimated abundance indices upon repeated counts along the selected transect, during the period of maximum activity (from mid April to the first days of June). We repeated the transect six times/year (Mezzasalma et al. 2016), for a total of 12 monitoring days in 2016–2017. Given the very low number of recaptures during the 2016–2017 period (only one *H. viridiflavus* recaptured, only one *N. helvetica* with two recaptures and two *N. helvetica* with one recapture each), it was not possible to analyse the recapture matrix with specific software as Capture, Mark and others. To estimate snake density, we have, therefore, considered the number of captured and recaptured snakes (divided per species) relative to the overall considered surface area, as in Rugiero et al. (2002).

Statistical data analyses

In our analyses, we used the large dataset of seven years of investigation distributed over a 21 years period. The Aesculapian snake (*Zamenis longissimus*) has been excluded from the analysis due to the very small sample size (only a few records in the last ten years). We applied a χ^2 on all the data that meet the test assumption, i.e. applicable when not more than 20% of all the cells have an expected frequency lower than five (Siegel 1980). Additionally, in short-term research, we performed a χ^2 to test possible fluctuations on the total number of captures per each species. We have then analysed average size variations between sampling years per each species using one-way two factors analysis of variance. Data were analysed using the software SPSS IBM (2011). All tests were considered significant if $P < 0.05$.

Results

Long term analysis

In the long-term analysis, we obtained a different pattern depending on the species considered (Fig. 1). *Hierophis viridiflavus* (8.7 ± 4.7 captures per year) was more common in the first years while it constantly decreased in the last period; on the contrary, *N. helvetica* (3.7 ± 4.2 captures per year) increased gradually in abundance; *V. aspis* (12.1 ± 7.4 captures per year) appeared relatively constant throughout the years. The total number of captures per species and year is reported in Table 1. Despite the exclusion of the years 1997 and 1999 (due to the absence of Grass snakes, that prevented the application of a χ^2 test), we obtained a significantly different distribution amongst years and species ($\chi^2 = 24.5$; df = 8; $P = 0.002$). From total snakes observed, per species, we obtained an average occurrence of 6.1 *H. viridiflavus*/100 m, 2.6 *N. helvetica*/100 m and 8.5 *V. aspis*/100 m.

Considering the pattern for each year, we recorded 0.3 to 1.7 *H. viridiflavus*/100 m, 0.1 to 1.0 *N. helvetica*/100 m, from 0.3 to 2.3 *V. aspis*/100 m. If we considered the available suitable surface of our transect, approximate-

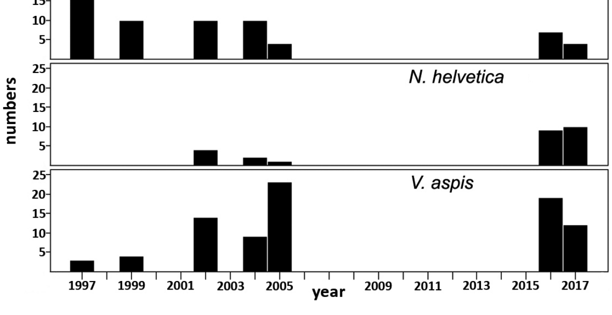

Figure 1. Distribution (as numbers) of captures per year of sampling from 1997 to 2017.

Table 1. Total number of captures per each species and sampling year.

Species	Sampling year							Total
	1997	1999	2002	2004	2005	2016	2017	
H. viridiflavus	17	10	10	10	4	7	3	61
N. helvetica	0	0	4	2	1	9	10	26
V. aspis	3	4	14	9	23	19	13	85

Table 2. Contingency table of captures per species and sampling year.

Period of the surveys	Species			Total
	H. viridiflavus	*N. helvetica*	*V. aspis*	
2016	7	9	19	35
2017	3	10	12	25
Total	10	19	31	60

Table 3. Population size variation from 1997 to 2017 (svl, in mm, ± 1 SD).

	H. viridiflavus	*N. helvetica*	*V. aspis*
1997	891.2 ± 169.6		341.7 ± 136.5
1999	1071 ± 44.3		540 ± 120.5
2002	793.4 ± 344.6	509.5 ± 128.6	275.4 ± 177.1
2004	861.0 ± 189.9	1053.5 ± 99.7	385.1 ± 148.0
2005	896.5 ± 100.7	918	356.4 ± 176.4
2016	925.4 ± 63.5	771.7 ± 105.1	572.3 ± 91.3
2017	663.3 ± 345.3	864.4 ± 192.2	425.3 ± 98.1

ly of 0.2 ha, we could infer a yearly density variability of 15–85 *H. viridiflavus*/ha, 5–50 *N. helvetica*/ha and 15–115 *V. aspis*/ha. If we consider a theoretic hectare of suitable habitat (i.e. five times our study area), we have to consider the ecotonal area bordering an hypothetical figure, as for simplicity of calculation, on a pentagon of 1000 m side or on a quadrate of 1250 m side: in this hypothetical scenario, our target habitat would represent the ecotones of a much larger area of ca. 172 or 156 ha, respectively. The estimated minimum-maximum densities should therefore be 0.09–0.49 or 0.1–0.54 *H. viridiflavus*/ha, 0.03–0.29 or 0.03–0.32 *N. helvetica*/ha and 0.09–0.67 or 0.1–0.74 *V. aspis*/ha, respectively.

Short term analysis

In 2016–2017, during 29 surveys, we captured, marked and recaptured 164 snakes, 60 of them individually marked. Only one *H. viridiflavus*, three *N. helvetica* and seven *V. aspis* were recaptured at least one time, only three *V. aspis* were recaptured at least three times, unfortunately preventing any further population analyses (e.g. recapture rate, survival etc.). The χ^2 test confirmed that no significant variation in population density occurred (χ^2 = 1.611; df = 2; P = 0.447) (Table 2).

In addition, population average body size varied significantly amongst years for each species (*H. viridiflavus*: F=2.244, df=6, P=0.037; *N. helvetica*: F=5.892, df=4, P=0.003; *V. aspis*: F=7.299, df=6, P < 0.0001) (Table 3). *Hierophis viridiflavus* differed especially in 1997, 1999, 2004 and 2017 with larger size in 1999 and smaller in 2017. *Natrix helvetica* differed amongst years, with larger size in 2017. *Vipera aspis* differed amongst all years, with larger size in 1999 and 2016 and smaller in 2002. Notably, body size variations recorded in 2002–2005 are referred to several newborns and juveniles with respect to the other years.

Monitoring protocol

For each year, we considered six randomly selected surveys to test the validity of monitoring techniques. During these 12 monitoring surveys, we captured 31 snakes in total, six *H. viridiflavus*, six *N. helvetica* and 19 *V. aspis*, that is on average 2.58 snakes/day (0.5 *H. viridiflavus*, 0.5 *N. helvetica* and 1.58 *V. aspis* per day).

Discussion

Long term analysis

Our study highlights that monitoring and analysing dynamics and ecology of snake species-community needs a long-lasting sampling effort to assess patterns of variation in species dynamics, in changes of population structure or even in ecology and phenology phenomena (Fitch 1999; Jäggi and Baur 1999; Gentilli et al. 2006; Rugiero et al. 2013). We observed even slight, but significant, variations in population size. In general, *H. viridiflavus*, *N. helvetica* and *V. aspis* represent common species of ecotonal areas of Mediterranean habitats, widely known to be generalist snakes (Filippi and Luiselli 2000). In our research, the average snake occurrence per year was 24.6 snake/yr (172 snakes in seven years), as in other European studies (Luiselli et al. 2011, *Natrix tessellata*, 21.5 snake/yr; Moreno-Rueda and Pleguezuelos 2007, *Malpolon monspessulanus*, 16.3 snakes/yr; Capula et al. 2014, *Hierophis viridiflavus*, 22 snakes/yr), but less than in others (Rugiero et al. 2002, *Hierophis viridiflavus*, 66 snakes/yr, *Zamenis longissimus*, 41 snakes/yr; Leliévre et al. 2013, *Hierophis viridiflavus*, 59 snakes/yr, *Zamenis longissimus*, 65 snakes/yr). Our data represent, however, low estimates when focusing on a single species, ranging from about four *N. helvetica* to 12 *V. aspis*, likely reflecting the carrying capacity of our small area. Our estimation from the hypothetical area with bordering ecotones, shows maximum densities of 0.49–0.54 *H. viridiflavus*, 0.29–0.32 *N. helvetica* and 0.67–0.74 *V. aspis* per ha. Rugiero et al. (2002) stated: "If we pool data for four habitat types, the mean density per year of *C. viridiflavus* adults was 2.32 ± 1.05 specimens × ha^{-1}, and the mean density of *E. longissima* adults was 1.34 ± 0.89 specimens × ha^{-1}." Analysing their Table 1 or also the number of marked *H. viridiflavus* (respectively 730 sighted or 511 marked snakes in 11 years, pooled from four different habitats of 20 ha each; see Rugiero et al. 2002, materials and methods and results), there emerges an almost certain error, with a much lower density estimation, that is from 0.83 to 0.58 *H. viridiflavus/ha,* respectively and on average ≈ 66 (66.4) snakes captured each year (error confirmed by the author, L. Luiselli, pers. com.). In the paper of Leliévre et al. (2013), of 1608 captured snakes in 13 years, 764 were *H. viridiflavus* (calculated from Table 1, p. 587). Those snakes were captured and recaptured in four different areas, whose overall surface (calculated according to the reference scale [as in Fig 1, p. 587], as area 1 = 11.27 ha; area 2 = 7.69 ha; area 3 = 6.93 ha; area 4 = 69.43 ha) is of 95.32 ha. From this overall surface, the estimated density for *H. viridiflavus* in north-western France (Chizé, Deux Sevres) in 13 years was ≈ 0.6 (0.62) snake/ha with, on average, ≈ 59 (58.8) snakes captured each year. Derived data from Rugiero et al. (2002) and Leliévre et al. (2013) provide results that are, however, much more comparable and in line with our findings, as is logical to be expected in the same species and in a very similar habitat structure, despite the fact that they were recorded in different climatic and geographical areas (Mediterranean climate in central Italy vs. Continental climate, in North-Western France). However, we should underline that, in our research, when comparing the observation of snakes throughout different years (Table 1), the patterns amongst species did differ significantly. *Hierophis viridiflavus* becomes less frequent as regards the number of individual snakes captured/recaptured. *Natrix helvetica*, on the contrary, was found for the first time in 2002 and seems to be increasing in number in the following years. *Vipera aspis* shows a relatively stable occurrence throughout the whole considered period, despite a relatively larger number of newborns captured and recaptured during 2017. In a long-lasting research (Rugiero et al. 2013), it was shown that climatic changes, namely global warming, induced an increase in delay in entering into hibernation and a decrease in aboveground activity of asp vipers (annual onset, *sensu* Rugiero et al. 2013), but no effect of such phenomenon on snake density has been recorded. We found some similarities in the occurrence patterns only in the impressive and longest snake community research published by Fitch (1999), despite studied species and their life-history traits not being directly comparable. Fitch (1999), in fact, reported strong density variations of *Coluber constrictor* in relation to the different fields and crops management, with increasing or decreasing herbaceous structure and small mammals' densities and, accordingly, increased or decreased snake densities.

Short term analysis

During 2016–2017, in our study area, the average body size of *H. viridiflavus* and *N. helvetica* did not change differently from the recorded significant variations in population abundance, underlining significant effects only on the snake occurrence, as found in Fitch (1999). More importantly, *V. aspis*, albeit stable throughout the long period, has significantly decreased its density and its body size in the last two years (we mostly recorded sub-adults and juveniles; data not shown). As many snake species are quite long-lived organisms (Madsen 1987; Flatt et al. 1997; Fornasiero et al. 2016) and some of our studied individuals have been found after 9–10 years since first capture, it may be logically hypothesised that the causes that have induced such population variability may have occurred several years before our analysis (Gentilli et al. 2006; Rugiero et al. 2013), most probably involving both *H. viridflavus* and *V. aspis*, whose adults' occurrence in the last two years was actually lower than reasonably expected. Only Jäggi and Baur (1999) were able to support robust hypotheses on *V. aspis* extinction at a local scale (as forest overgrowing increased humidity and decreased sun exposition) and even our data do not provide information about any possible ongoing extinction process. Prey availability and/or predators presence was not recorded for such a long period (see Saviozzi and Zuffi 1997), thus, as far as we are concerned, it is not easy to formulate any valid hypotheses for what we recorded. Alternatively, we can hypothesise that a fluctuation in snake composition, like the one we found, may represent a natural pattern, whose slight variations are, on average, difficult to detect over a few years, but are quite common and easy to detect in long term research (Fitch 1999; Jäggi and Baur 1999; Sullivan 2000; Rugiero et al. 2002; Moreno-Rueda and Pleguezuelos 2007; Pringle et al. 2009; Leliévre et al. 2013). Actually, natural habitats of and around our study area (i.e. wooded area surface, ecotonal structure) have not shown any evident or measurable change during the period, as emerged from historical vs. recent aerial photographs (Google Earth images), supporting our hypothesis of no habitat effect on snakes dynamics, different from what was observed in northern Italy (Gentilli et al. 2006) and in Switzerland (Jäggi and Baur 1999). The appearance of *N. helvetica* may represent a new colonisation of the area, given several humid and marshed areas and canals very close to our study area, the transformation of which should be studied in future research.

Monitoring protocol

Finally, we are therefore confident that the monitoring system protocol of the Italian Environment Ministry (Stoch and Genovesi 2016) is a practical, highly informative and easy to apply tool to be carried out by professional herpetologists, even if it would deserve quite a large effort in the field before assessing true population dynamics.

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