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First data on the consumed prey by *Speleomantes italicus* from the Republic of San Marino

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Abstract. We here present the first study on the consumed prey by an epigean population of the Italian cave salamander (*Speleomantes italicus*) from the Republic of San Marino. Using the harmless technique of stomach flushing, we aimed to provide the first data on the diet and on the degree of individual specialization that characterizes the studied population. We performed two-night surveys (one in autumn 2022 and one in spring 2023) in which we opportunistically searched for salamanders in forested areas and dry walls. Captured salamanders were sexed, weighed, photographed, measured and stomach flushed. Overall, we inspected the stomach contents of 67 individuals, recognizing 1,018 prey items belonging to 28 different prey categories. The examined population was mainly composed by generalist individuals, and no differences in number and diversity of prey consumed were observed among salamanders. Contrarily to what has been observed in other *Speleomantes* species, flying prey provided a minor contribution to the diet of the investigated population. Our study produced the first information on the diet of *Speleomantes italicus* from the Republic of San Marino, representing the starting point for future assessments on the dietary habits of this specific population.

Keywords. Diet, *Hydromantes*, Plethodontidae, salamander, stomach flushing, trophic niche, Urodela.

European plethodontid salamanders (genus *Speleomantes*) are eight allopatric species mainly distributed in Italy and in a small area of the French Provence (Lanza et al., 2006); among the mainland species, *S. italicus* also occurs in the Republic of San Marino (Casali et al., 2005). *Speleomantes* are facultative cave-dwellers inhabiting different typologies of subterranean environments (e.g., caves, mines), but also forested areas and even dry walls (Costa et al., 2016; Lanza et al., 2006; Manenti, 2014), where suitable microclimatic conditions (relatively cold temperature and high humidity) occur (Ficetola et al., 2018).

Speleomantes are generalist predators feeding on a wide number of invertebrate species (Lunghi et al., 2018a; Salvidio, 1992; Vignoli et al., 2006). Within subterranean environments, *Speleomantes* can forage throughout the year (Lunghi et al., 2022a), while in surface ones they are generally active only during favorable seasons (e.g., in spring and autumn) (Costa et al., 2016;

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Salvidio et al., 2012). The main method used for studying the dietary habits of the genus is the stomach flushing (Salvidio, 1992). The use of stomach contents therefore turned out to be an optimal and sustainable method allowing to assess *Speleomantes* trophic niche, but also to evaluate the degree of diet specialization that individuals have within populations (Lunghi et al., 2020a; Lunghi et al., 2020d; Salvidio et al., 2015; 2017). However, this methodology was never adopted to study the trophic niche of *Speleomantes* populations occurring in the Republic of San Marino.

We here present the first data on the dietary habits of a population of *S. italicus* occurring in the Republic of San Marino, focusing on the diversity of prey consumed and on the diet specialization of individuals. We selected a population occurring within an urbanized area, hypothesizing that the related environmental conditions may cause a divergence of dietary habits compared to populations occurring in natural areas.

We performed two-night surveys in the Republic of San Marino, searching for individuals of *Speleomantes italicus* in a patched forested area of broad-leaved trees, and inside dry walls occurring within an urbanized area. The first survey was performed on 21st November 2022 between 8:00 p.m. and 0:00 a.m. (minimum air temperature Tmin 3.1 °C, maximum air temperature Tmax 11.2 °C, precipitations 1.5 mm), while the second one on 7th April 2023 between 9:00 p.m. and 1:00 a.m. (Tmin 4.7 °C, Tmax 11.6 °C, precipitations 0 mm) (climatic data are retrieved from https://www.3bmeteo.com). All operators worked using disposable gloves and used clean and disinfected instruments. Salamanders were opportunistically searched and captured individuals were processed as follow. Adult males were recognized by checking the presence of male secondary sexual characters (i.e., mental gland) (Lanza et al., 2006). Adult females and juveniles were later distinguished on the basis of snout-vent length (SVL); individuals with SVL ≥ 50 mm were considered adult females, while the other juveniles (Lunghi, 2022). Salamanders were then weighed on a digital scale (0.01 g) and placed on the portable studio to shoot a picture which will be used to estimate individuals SVL in the lab using the ImageJ software (https://imagej.nih.gov/ij) (Lunghi et al., 2020c). All pictures were visually checked in order to individually recognize salamanders using their dorsal pattern (Lunghi et al., 2019); no individual was captured twice. Finally, we performed stomach flushing on salamanders (discarding juveniles with SVL < 40 mm) to inspect the residuals of their last foraging activity. Stomach contents were preserved in 70% EtOH until their recognition. Prey items were counted and recognized following (Lunghi et al., 2018a). Briefly, prey items were generally recognized at order level, distinguishing between larval and adult stages when morphology and ecology of the two life stages strongly diverge. For two groups (Coleoptera and Hymenoptera) we were able to reliably recognize prey items from a specific family, respectively Staphylinidae and Formicidae; these families were used as independent prey categories (Lunghi et al., 2018a).

We pooled together data from the two seasons as the high divergence of the number of recognized prey between seasons (1:10) would affect the reliability of such comparison. We used Generalized Linear Mixed Models (GLMMs) (Douglas et al., 2015) to evaluate whether salamanders consumed a larger number or higher diversity (Shannon index) of prey on the basis of their size (SVL) or sex (adult males, adult females, juveniles). GLMMs were built using the log-transformed number of consumed prey or the Shannon index as dependent variable, while the SVL and sex were used as independent variables; date of survey was used as random factor. Likelihood ratio test was used to assess the significance of variables in GLMMs (Kuznetsova et al., 2016). We then estimated the degree of individual diet specialization (IS) (Zaccarelli et al., 2013). We calculated IS and transformed using the index (V = 1 – IS) proposed in Bolnick et al., (2007), where values tending to 1 indicate a high degree of individual diet specialization. Bootstrapping (repeated 9999 times) was used to test whether the observed index of individual diet specialization diverged significantly from a scenario in which all individuals randomly chose their prey. To avoid over estimations of IS, the dataset used in this analysis only included individuals from which at least 3 prey items were recognized.

We inspected the stomach contents of 67 individuals of *Speleomantes italicus* (46 from autumn and 21 from spring) including 26 adult females, 29 adult males and 12 juveniles (Table S1). Only 2 individuals from autumn had empty stomach. We overall recognized 1,018 prey items (average ± SD per individual; 15.66 ± 13.23) belonging to 28 groups of prey (Table 1).

During autumn, the captured individuals consumed 921 prey items belonging to 27 categories, where three groups of prey (Diptera larvae, Entomobryomorpha, and Hemiptera) accounted for 62.48% of the consumed prey. In spring the captured salamanders consumed 97 prey items belonging to 17 prey categories, where two groups (Diptera and Araneae) accounted for 44.33% of the consumed prey. These proportions only considered prey categories with relative importance > 10% (Table 1). The number of consumed prey was not affected by both salamander size ($F_{1,60}$ = 1.72, P = 0.195) and sex ($F_{2,60.02}$ = 0.11, P = 0.897). Similar results were obtained for the diversity of consumed prey (size, $F_{1,60}$ = 0.76, P = 0.387;

Table 1. List of the prey residuals recognized from stomach contents of *Speleomantes italicus* from the Republic of San Marino. In brackets, the relative importance (%) of each category of consumed prey calculated for each season.

Prey order	Number of recognized prey (relative importance, %)	
	Autumn 2022	Spring 2023
Pulmonata	24 (2.61)	0
Sarcoptiformes	50 (5.43)	8 (8.25)
Trombidiformes	1 (0.11)	1 (1.03)
Araneae	25 (2.71)	14 (14.43)
Pseudoscorpiones	33 (3.58)	3 (3.09)
Opiliones	11 (1.19)	1 (1.03)
Lithobiomorpha	6 (0.65)	1 (1.03)
Geophilomorpha	7 (0.76)	0
Scolopendromorpha	1 (0.11)	0
Polydesmida	37 (4.02)	1 (1.03)
Isopoda	8 (0.87)	2 (2.06)
Symphypleona	14 (1.52)	2 (2.06)
Poduromorpha	6 (0.65)	0
Entomobryomorpha	221 (24)	7 (7.22)
Blattodea	0	1 (1.03)
Psocodea	1 (0.11)	0
Hemiptera	121 (13.14)	8 (8.25)
Hymenoptera	9 (0.98)	0
Hymenoptera_Formicidae	24 (2.61)	6 (6.19)
Coleoptera	12 (1.3)	4 (4.12)
Coleoptera_Staphylinidae	10 (1.09)	0
Coleoptera_larva	12 (1.30)	0
Lepidoptera	2 (0.22)	0
Lepidoptera_larva	29 (3.15)	1 (1.03)
Diptera	24 (2.61)	29 (29.90)
Diptera_larva	231 (25.08)	8 (8.25)
Dermaptera	1 (0.11)	0
*Speleomantes*_skin	1 (0.11)	0
Total	921	97

sex, $F_{1,60.03} = 0.3$, $P = 0.742$). Overall, 11 individuals consumed < 3 prey items; these individuals were excluded from diet specialization analysis. The studied population of *S. italicus* showed a very low proportion of specialized individuals ($V = 0.28$, $P < 0.001$).

This study provides the first information on the diet of *Speleomantes italicus* from the Republic of San Marino. The unfavorable climatic conditions (i.e., dryness) occurring during our spring survey may have negatively affected the activity of both predators (salamanders) and prey (invertebrates), limiting our data collection and therefore hampering the possibility to provide meaningful and robust comparisons between the two seasons. In the future, multiple seasonal surveys and data collection on prey availability may allow to produce a more complete knowledge on the trophic niche for this population and evaluate the potential effect of local microclimate on prey availability (Centomo et al., 2023; Lunghi et al., 2018b; Salvidio, 1992).

All the 28 prey categories recognized from this population were already known for the species (Lunghi et al., 2022b), although divergences in the relative proportion can be observed. The first evident divergence is the proportion of consumed flying prey. In the present population flying prey (Ephemeroptera, Hymenoptera, Mecoptera, Plecoptera, Lepidoptera, Diptera) only accounted for less than 19% of the overall consumed prey (Table 1), with a relatively low increase in their proportion in spring (Table 1). This is quite interesting as *Speleomantes* seems to have a preference for flying prey independently from the habitus of the considered population (i.e., epigean or subterranean) (Roth 1982), hypothesis supported by the large proportion of this type of prey within *Speleomantes* diet (71.5% in Lunghi et al., 2018a; 94% in Lunghi et al., 2021; 52.7% in Lunghi et al., 2022b; 85% in Salvidio et al., 1994; 53% in Vignoli et al., 2006; but see Salvidio, 1992). Furthermore, *Speleomantes* are known to increase the consumption of this prey type at the beginning of the warm season, particularly when the abundance of Diptera in caves is higher (Lunghi et al., 2020b; 2022a). However, we must remark that our data is strongly biased towards autumn, a season in which the consumption of non-flying prey generally increases compared to spring (Lunghi et al., 2018b; 2022a); indeed, further samplings are needed to clarify this unexpected result. Another curiosity emerging from this dataset is the very large proportion of consumed Entomobryomorpha, especially in autumn, where they represented 24% of the total consumed prey (Table 1). Although Entomobryomorpha are relevant components of terrestrial ecosystems and constitute a significant proportion of the animal biomass (Handschin 1955; Kampichler and Bruckner 2009), the reason for this high proportion is difficult to hypothesize (especially at this level of taxonomic rank) and probably lies in the composition of the prey available in these survey periods.

The sampled population was characterized by a high proportion of generalist individuals ($V = 0.28$), characteristic also supported by the results of GLMMs analyses, where neither sex nor size did affect both number and diversity of consumed prey. A high proportion of diet specialization has been observed in spring within an epigean population of *S. italicus*, where the population seems to not be in competition with the sympatric *Salamandra salamandra* (Lunghi et al., 2022c). A high

proportion of specialists may arise due to competition, or because the wide trophic availability allows individuals to specialize on the preferred prey (Araújo et al., 2011). If we consider only the data collected in spring (Table 1), individuals from the Republic of San Marino consumed 17 prey categories while individuals from the other epigean population 35 (Lunghi et al., 2022c), supporting the hypothesis that a wider trophic availability may be responsible for diet specialization of individuals. The two populations strongly diverge in the type of habitat they occupy, one located within an urbanized area and the other in a fully forested area, meaning that probably the area interested by less urbanization may show higher prey diversity (Buczkowski and Richmond 2012; Chapman 2003). This is an interesting hypothesis that deserves to be investigated in the future.

With this study, we provided the first information on the dietary habits of *Speleomantes italicus* from the Republic of San Marino. Our results should be considered the starting point to further investigate the trophic niche of this *Speleomantes* population and to test the multiple hypotheses raised by this study.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-262 9.unipv.it/webshi/appendix/index.html> manuscript number 14748.

REFERENCES

Araújo, M.S., Bolnick, D.L., Layman, C.A. (2011): The ecological causes of individual specialisation. Ecol. Lett. **14**: 948-958.

Bolnick, D.I., Svanbäck, R., Araújo, M.S., Persson, L. (2007): Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proc. Natl. Acad. Sci. USA **104**: 10075-10079.

Buczkowski, G., Richmond, D.S. (2012): The Effect of Urbanization on Ant Abundance and Diversity: A Temporal Examination of Factors Affecting Biodiversity. PLoS ONE **7**: e41729.

Casali, S., Suzzi Valli, A., Busignani, G., Tedaldi, G. (2005): Costumi arboricoli di *Speleomantes italicus* (Dunn, 1923) nella Repubblica di San Marino. Annali del Museo Civico di Storia Naturale Giacomo Doria Genova **97**: 145-152.

Centomo, E., Roner, L., Salvatori, M., Pedrini, P., Romano, A. (2023): Rare and hungry: feeding ecology of the Golden alpine salamander, an endangered amphibian in the Alps. Animals **3**: 2135.

Chapman, M.G. (2003): Paucity of mobile species on constructed seawalls: effects of urbanization on biodiversity. Mar. Ecol. Prog. Ser. **264**: 21-29.

Costa, A., Crovetto, F., Salvidio, S. (2016): European plethodontid salamanders on the forest floor: local abundance is related to fine-scale environmental factors. Herpetol. Conserv. Biol. **11**: 344-349.

Douglas, B., Maechler, M., Bolker, B., Walker, S. (2015): Fitting Linear Mixed-Effects Models using lme4. J. Stat. Softw. **67**: 1-48.

Ficetola, G.F., Lunghi, E., Canedoli, C., Padoa-Schioppa, E., Pennati, R., Manenti, R. (2018): Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. Sci. Rep. **8**: 10575.

Handschin, E. (1955): Considérations sur la position systématique des Collemboles. Mem. Soc. R. Entomol. Belgique Jublaire, 1955(Vingt-Septième): 40-53.

Kampichler, C., Bruckner, A. (2009): The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. Biol. Rev. **84**: 375-389.

Kuznetsova, A., Brockhoff, B., Christensen, H.B. (2016): lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-2.9 DOI: www.r-project.org

Lanza, B., Pastorelli, C., Laghi, P., Cimmaruta, R. (2006): A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). Atti Mus. civ. stor. nat. Trieste **52**: 5-135.

Lunghi, E., Cianferoni, F., Ceccolini, F., Mulargia, M., Cogoni, R., Barzaghi, B., Cornago, L., Avitabile, D., Veith, M., Manenti, R., Ficetola, G.F., Corti, C. (2018a): Field-recorded data on the diet of six species of European *Hydromantes* cave salamanders. Sci. Data **5**: 180083.

Lunghi, E., Cianferoni, F., Ceccolini, F., Veith, M., Manenti, R., Mancinelli, G., Corti, C., Ficetola, G.F.

(2018b): What shapes the trophic niche of European plethodontid salamanders? PLoS ONE **13**: e0205672.

Lunghi, E., Romeo, D., Mulargia, M., Cogoni, R., Manenti, R., Corti, C., Ficetola, G.F., Veith, M. (2019). On the stability of the dorsal pattern of European cave salamanders (genus *Hydromantes*). Herpetozoa **32**: 249-253.

Lunghi, E., Cianferoni, F., Ceccolini, F., Zhao, Y., Manenti, R., Corti, C., Ficetola, G.F., Mancinelli, G. (2020a): Same diet, different strategies: variability of individual feeding habits across three populations of Ambrosi's cave salamander *(Hydromantes ambrosii*). Diversity **12**: 180.

Lunghi, E., Ficetola, G.F., Zhao, Y., Manenti, R. (2020b): Are the neglected Tipuloidea crane flies (Diptera) an important component for subterranean environments? Diversity **12**: 333.

Lunghi, E., Giachello, S., Manenti, R., Zhao, Y., Corti, C., Ficetola, G.F., Bradley, J.G. (2020c): The post hoc measurement as a safe and reliable method to age and size plethodontid salamanders. Ecol. Evol. **10**: 11111-11116.

Lunghi, E., Manenti, R., Cianferoni, F., Ceccolini, F., Veith, M., Corti, C., Ficetola, G.F., Mancinelli, G. (2020d): Interspecific and inter-population variation in individual diet specialization: do environmental factors have a role? Ecology **101**: e03088.

Lunghi, E., Cianferoni, F., Giachello, S., Zhao, Y., Manenti, R., Corti, C., Ficetola, G.F. (2021): Updating salamander datasets with phenotypic and stomach content information for two mainland *Speleomantes*. Sci. Data **8**: 150.

Lunghi, E. (2022): Doubling the lifespan of European plethodontid salamanders. Ecology **103**: e03581.

Lunghi, E., Cianferoni, F., Corti, C., Zhao, Y., Manenti, R., Ficetola, G.F., Mancinelli, G. (2022a): The trophic niche of subterranean populations of *Speleomantes italicus*: a multi-temporal analysis. Sci. Rep. **12**: 18257.

Lunghi, E., Corti, C., Biaggini, M., Merilli, S., Manenti, R., Zhao, Y., Ficetola, G.F., Cianferoni, F. (2022b): Capture-mark-recapture data on the strictly protected *Speleomantes italicus*. Ecology **103**: e3641.

Lunghi, E., Corti, C., Biaggini, M., Zhao, Y., Cianferoni, F. (2022c): The trophic niche of two sympatric species of salamanders (Plethodontidae and Salamandridae) from Italy. Animals **12**: 2221.

Manenti, R. (2014): Dry stone walls favour biodiversity: a case-study from the Appennines. Biodivers. Conserv. **23**: 1879-1893.

Roth, G. (1982): Responses in the optic tectum of the salamander *Hydromantes italicus* to moving prey stimuli. Exp. Brain Res. **45**: 386-392.

Salvidio, S. (1992): Diet and food utilization in a rock-face population of European plethodontid *Speleomantes ambrosii* (Amphibia, Caudata, Plethodontidae). Vie et Milieu **42**: 35-39.

Salvidio, S., Latters, A., Tavano, M., Melodia, F., Pastorino, M.V. (1994): Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. Amphib-Reptilia **15**: 35-45.

Salvidio, S., Romano, A., Oneto, F., Ottonello, D., Michelon, R. (2012): Different season, different strategies: feeding ecology of two syntopic forest-dwelling salamanders. Acta Oecol. **43**: 42-50.

Salvidio, S., Oneto, F., Ottonello, D., Costa, A., Romano, A. (2015): Trophic specialization at the individual level in a terrestrial generalist salamander. Can. J. Zool. **93**: 79-83.

Salvidio, S., Pasmans, F., Bogaerts, S., Martel, A., van de Loo, M., Romano, A. (2017): Consistency in trophic strategies between populations of the Sardinian endemic salamander *Speleomantes imperialis*. Anim. Biol. **67**: 1-16.

Vignoli, L., Caldera, F., Bologna, M.A. (2006): Trophic niche of cave populations of *Speleomantes italicus*. J. Nat. Hist. **40**(29-31): 1841-1850.

Zaccarelli, N., Bolnick, D.I., Mancinelli, G. (2013): RInSp: an R package for the analysis of individual specialization in resource use. Methods Ecol. Evol. **4**: 1018-1023.